Asian Journal of Physics
Vol. 31, No 2 (2022) 227-238
Available on: www.asianjournalofphysics.com

ap

Ultrafast vibrational and electronic relaxation of carotenoids investigated with Femtosecond Stimulated Raman Spectroscopy

E Ragnoni[1], T M Kardaś[2], A Lapini[1,3], P Foggi[1,4], R Righini[1] and M Di Donato[1,5]

[1]*LENS (European Laboratory for non Linear Spectroscopy, via N. Carrara 1, 50019 Sesto Fiorentino (FI), Italy*

[2]*Fluence sp. z o.o., ul. Kolejowa 5/7, 01-217 Warsaw, Poland*

[3]*Department of Chemistry, Life Science and Sustainability, University of Parma, Parco Area delle Scienze, 11/a, 43124 Parma*

[1]*Department of Chemistry, Biology and Biotechnology, via Elce di Sotto, 8 - 06123 Perugia (PG) – Italy*

[5]*ICCOM-CNR, via Madonna del Piano 10-12, 50019 Sesto Fiorentino (FI), Italy*

This paper reviews the main results obtained by using the technique of Femtosecond Stimulated Raman Spectroscopy (FSRS) to study the photophysics of carotenoids. After a brief presentation of the technique and of its practical implementation, its potentialities in disentangling the very fast relaxation processes occurring upon light absorption in carotenoids are presented. It is shown that the recourse to FSRS allows to clarify the timescale of vibrational energy relaxation in the electronic excited states of these molecules, and to clearly identify the nature of the excited states involved in the photophysics of several naturally occurring pigments, both in solution and when embedded in photosynthetic proteins.

Keywords: Carotenoids, Femtosecond Stimulated Raman Spectroscopy, Internal Vibrational Relaxation

1 Introduction

Carotenoids are pigments contained in plants, algae and photosynthetic proteins, and are responsible for the bright yellow-orange color of multiple vegetables and fruits. Carotenoids can have different functions, mostly connected with their antioxidant properties. In photosynthetic proteins carotenoids have both a photoprotecting role, since they can react with singlet oxygen and harmful radical species, and a light harvesting role, being able to absorb in the green region of the solar spectrum and transfer energy to chlorophylls, which instead mostly absorb in the blue and red regions.

Because of their important role in photosynthesis, the photophysical processes connected to the relaxation of the excited states of carotenoids have been the object of multiple spectroscopic and theoretical investigations [1-6].

The excited state manifold of carotenoids is generally described taking as a reference that of linear polyenes [7], and considering that the molecules possess a pseudo-C_{2h} symmetry. According to this description, the ground state S_0 will have an A_g^- symmetry, the same as the first excited state S_1, making the $S_0 \rightarrow S_1$ transition prohibited because of symmetry selection rules. The following excited state S_2 is instead classified as B_u^+, so that a transition from S_0 is possible. The first allowed electronic transition for carotenoids is thus $S_0 \rightarrow S_2$, which gives rise to the characteristic absorption band of these molecules in the 480-520 nm spectral range. A simplified energetic scheme for the low lying excited states of carotenoids is reported in Fig 1, together with the structure of several carotenoids discussed in this work.

Corresponding author
e mail: didonato@lens.unifi.it (M Di Donato)

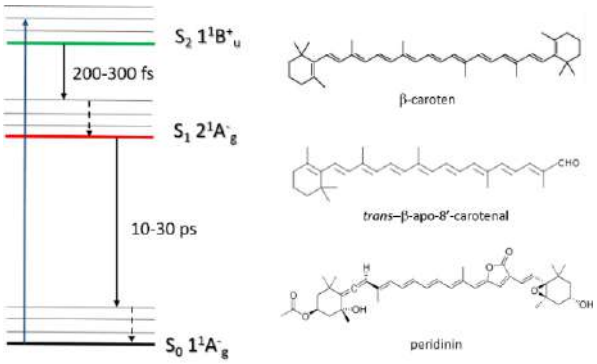


Fig 1. Energy levels of low lying electronic states of carotenoids and structure of several carotenoids analyzed in this work.

The excited state relaxation can be described, as a first approximation, in terms of a very fast $S_2 \rightarrow S_1$ internal conversion (IC), occurring on the sub-ps timescale, and a slower $S_1 \rightarrow S_0$ relaxation, whose rate constant can depend on multiple factors, such as the polyene length, the nature of the external medium or the presence of substituents on the polyene chain. Multiple time resolved spectroscopic studies have evidenced that this simple relaxation scheme can not be appropriate to correctly describe the photophysics of carotenoids, as, due to the presence of different substituents on the polyene chain, the symmetry rules are not strictly applicable. These studies have thus invoked the presence of intermediate dark states, whose nature is still highly debated, in order to correctly interpret the relaxation dynamics of these pigments [8-10].

A particularly interesting case is that of carotenoids presenting an asymmetric carbonyl group linked to their polyene chain, such as carotenals (carotenoids containing an aldehyde functional group) that are often found in natural systems. Examples of naturally occurring carbonyl carotenoids are for instance peridinin, present in the Peridinin Chlorophyll Protein (PCP), or fucoxanthin, which is present in brown algae. One of the most peculiar properties of carbonyl carotenoids is that their excited state lifetime becomes highly dependent on solvent polarity [11-15], shortening significantly as the polarity of the solvent increases. This behavior is usually explained considering that the presence of the carbonyl can induce a significant charge rearrangement in the excited state, conferring an intramolecular charge transfer (ICT) character to the low-lying energy levels. Although this explanation is generally accepted in the literature, discussions are still open concerning the exact nature of the ICT state, its role in the photodynamics and the mechanism of its formation [1,5,11,16,17].

In this context, the recourse to time resolved spectroscopic techniques for the analysis of the infrared/Raman activity of the molecule with high temporal resolution can be extremely useful. Compared to visible absorption, the infrared and/or Raman spectrum of a molecule can indeed disclose much more extending structural information, being the vibrational bands directly connected with the nature and strength of the chemical bonds. Using time resolved IR/Raman spectroscopic techniques it is thus possible to follow the structural modifications of a sample in the excited state, gathering, at the same time information about the electronic redistribution following light absorption. Indeed, it is well known that molecular vibrations are highly sensitive to electronic rearrangements, and shift in their peak positions are expected for instance in case of charge localization on one part of the molecule, or polarization of a chemical bond because of interaction with a solvent. This information can be useful in the analysis of the excited state relaxation of carotenoids, in order to understand what structural variations accompany excited state formation or how the solvent can influence the photodynamics in case of carbonyl carotenoids. Numerous studies have appeared in the literature, in which the photophysics of carotenoids was studied using both infrared and Raman time resolved methods [5,9,11,18-22]. In particular, soon after its development, the technique of femtosecond

stimulated Raman spectroscopy (FSRS), has immediately appeared as extremely suited to clarify the excited state relaxation mechanism of these molecules, since the multiple C=C and C-C bonds present in the polyene chain of carotenoids have an intense Raman activity [18,23,24]. The aim of this paper is that of giving an overview of the potentiality of the FSRS technique and of summarizing the main information that our and other groups have retrieved using this method in the analysis of the excited state relaxation of different type of carotenoids.

The paper is organized as follows: after a description of the technique and its practical implementation, several previous studies will be reviewed, starting from the initial application to β-carotene and then moving towards biologically relevant carbonyl carotenoids, such as trans-β-apo-8'-carotenal (shortly apocarotenal), which is a precursor of vitamin A, peridinin, fucoxanthin and carotenoids contained in the antenna protein LHCII.

2 Result and Discussion

FSRS

Time-Resolved Femtosecond Stimulated Raman Scattering (FSRS) [25,26], is a particularly interesting technique, extremely suited for studying the mechanisms of internal conversion and vibrational energy redistribution, because of its high temporal resolution. Initial implementations of this technique date back to the mid-1990s-early 2000s [25,27,28], but it is only after 2003 that significant advances in terms of temporal resolution have been gained, thanks to the developments introduced by the group of R Mathies [24]. Excellent reviews by the same authors have appeared, describing in details both the experimental implementation and theoretical background of this spectroscopic method [26,29,30]. Here, we will review the most important aspects of this technique.

In order to obtain Stimulated Raman Scattering, two coherent beams at frequencies ω_p and ω_s, (the index p and s indicating the pump and Stokes (probe) beams), are sent to the sample. If the sample has a vibration at frequency $\omega_n = \omega_p - \omega_s$, the Raman transition will be enhanced, causing an attenuation of the pump beam and a gain of the Stokes beam. In FSRS, in order to study samples in the excited state, a third beam, referred to as actinic pump, is introduced, whose wavelength is tuned as to be resonant with an electronic transition of the sample, thus promoting part of the sample's population into an electronic excited state. The Raman spectrum is collected using two simultaneous pulses: a long (~1ps) narrowband Raman pump and a short (tens of fs) broadband Raman probe. The Stimulated Raman effect produces an amplification of the probe at all the vibrational frequencies that are stimulated by the Raman pump. Thus, sharp amplified vibrational peaks appear over the broadband spectral profile of the probe on both the Stokes and anti-Stokes sides. Furthermore, being the sample electronically excited by the actinic pump, both the stimulated Raman spectra of the ground and excited states are gathered, as a function of the delay with respect to the excitation. The time resolution of the technique can be very high. In principle, it only depends on the cross-correlation between the probe and actinic pump pulses, which, if using femtosecond amplified lasers and upon suitable compression, can be as short as a few tens of femtoseconds. In practice however, ultrashort pulses introduce different artefacts around time zero, such as the *"perturbed free induction decay"* (PFID) and possibly hot luminescence [27]; as a result the time resolution is generally determined by the product of the PFID and Raman probe duration. The spectral resolution of the experiment is determined by the spectral width of the Raman pump pulse, making it possible for this technique to achieve both good spectral and temporal resolution.

Implementation

A schematic representation of the setup used for the measurements on apocarotenal and peridinin described in the following section is depicted in Fig 2. This setup has been previously described in reference [18].

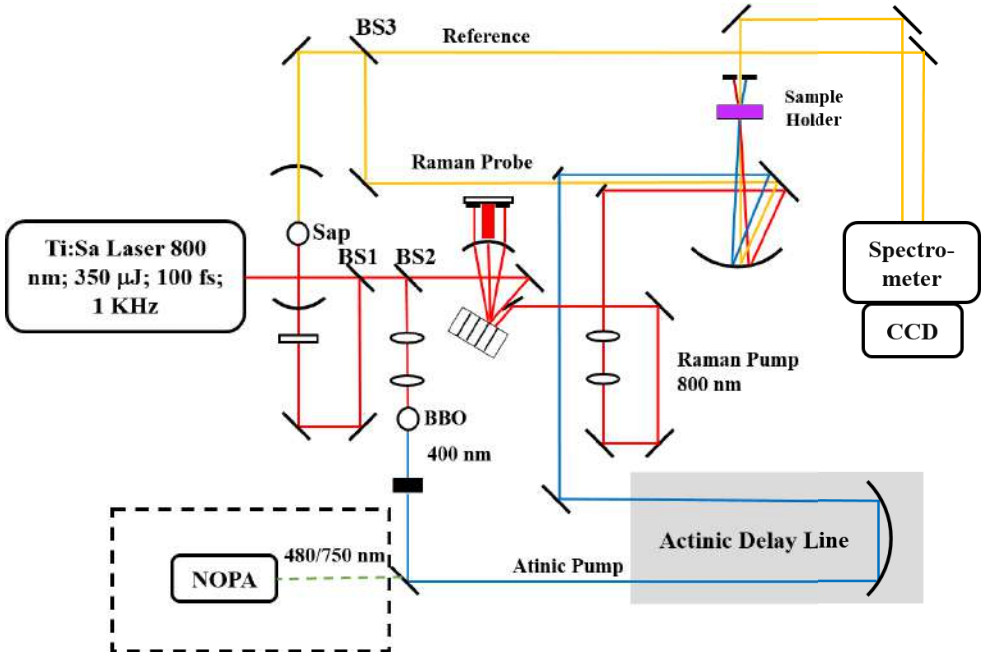


Fig 2. Schematic representation of a typical FSRS setup.

The setup is based on an integrated ultrafast Ti:Sapphire oscillator (Micra, Coherent Inc) and femtosecond regenerative amplifier (Legende Elite, Coherent Inc), capable of producing 40 fs pulses with a central wavelength of 800 nm, at 1kHz repetition rate. After entering the setup, the beam is divided in two portions by a 7% reflecting beamsplitter (BS1). The reflected portion is focused on a 1 mm Sapphire window to generate a white light continuum used as the Raman probe. The supercontinuum beam is then split by a 50% broadband beamsplitter (BS3) into probe and reference beams. The probe polarization is controlled with an 800 nm wave-plate. The remaining portion of the incoming beam is used to generate both the Raman and the actinic pump. A beam splitter (BS2) inserted in the optical path of the fundamental radiation transmitted by BS1, allows to separate it into two portions, used respectively for Raman and actinic pump generation. For the experiments performed on apocarotenal and peridinin described in this work, the wavelength of the actinic pump was set at 400 nm, which is obtained by frequency doubling of the 800 nm laser fundamental in 1 mm BBO crystal. Alternative pump wavelengths could be generated by using a Non-Collinear Optical Parametric Amplifier (NOPA), not implemented in the setup described here, which would allow the generation of actinic pump pulses in the visible region, tunable in the 470-750 nm range. The actinic pump is directed towards a movable delay line, which allows to vary the delay between the actinic pump and the probe pulses up to 1.5 ns. The Raman pump pulses are obtained by narrowing the spectrum of the 800 nm beam to about 25 cm^{-1} (corresponding to the pump pulse duration of 760 fs) through a 4f grating compressor with a mask in the Fourier plane [31,32]. Alternative schemes to obtain the Raman pump have been used, as described in ref. [30], the simplest one being spectral filtering of the 800 nm laser radiation with a narrowband filter. The delay time between Raman pump and probe is set to zero by finely adjusting the pathway of the Raman pump using a translator stage.

All the beams are then focused on the sample with a 150 mm concave mirror. The spot sizes of all the beams are controlled to get 80 mm in diameter at the sample. Telescopes are used to control the actinic and Raman pump beams, while the probe is controlled by adjusting the position of a collimating lens placed after the sapphire plate. The energy of the actinic pump pulse and the Raman pump were set to 200 nJ and

2.5 μJ, respectively for the described experiments. After passing the sample, the probe and reference beams are focused at the entrance of a 25 cm Jobin Yvon monochromator (HR 250), equipped with an homemade detector consisting of two CCD linear arrays (S8380-512Q, Hamamatsu). A chopper is inserted in both the Raman pump and actinic pump arms synchronized, respectively, to half the detector's acquisition frequency and to 500 Hz. The stimulated Raman scattering signal (S) is obtained as:

$$S = \frac{I_{pr}^{on}}{I_{ref}^{on}} \Big/ \frac{I_{pr}^{off}}{I_{ref}^{off}}$$

where I_{pr} and I_{ref} are the intensities of the probe and reference beams, while the apex *on* and *off* refer to the Raman pump. A background signal is previously measured and subtracted to the numerator and denominator of the equation. The stimulated Raman gain is calculated as $g = 1/d*\log(S)$, where d is the sample optical path. This signal contains contributions from both ground and excited states of the examined molecular system and from the solvent. The transient SRS signal is then obtained as the difference between the Raman gain in the presence and absence of the actinic pump, and by subtracting the solvent contribution.

In most cases, the baseline of the time resolved SRS is not flat, possibly due to transient absorption induced in the sample by the actinic pump and to modulation of the probe spectrum caused by the Raman pump. The correction is generally implemented by subtracting a polynomial function fitted to the raw baseline, excluding the regions where vibrational bands are observed.

FSRS spectra of carotenoids

The first application of FSRS to study the dynamics of carotenoids has concerned with the analysis of the excited state relaxation of β-carotene [24]. The ground state SRS of β-carotene presents three main absorption bands: a C=C double bond stretching band at 1525 cm^{-1}, a C-C single bond stretching band at 1159 cm^{-1} and a C-H methyl rocking band at 1007 cm^{-1}. As previously inferred from picosecond Raman measurements [33], upon excitation to S_2 and fast internal conversion to S_1, shifts are observed in the Raman bands compared to the ground state absorptions. In particular, the C=C double bond absorption significantly upshifts to about 1760 cm^{-1} because of the vibronic coupling between the S_0 and S_1 states. The other bands undergo only minor shifts upon excitation. In their initial study, McCamant *et al* did not resolve any Raman band assignable to the S_2 excited state, whose lifetime is extremely short [24]. By analyzing the kinetics of the rise and decay of the S_1 C=C stretching band, they were however able to disentangle the excited state relaxation of the molecule and the associated vibrational dynamics. The transient data were fitted with a sequential decay scheme, considering a very fast $S_2 \rightarrow S_1$ internal conversion, whose rate was fixed at 160 fs, leading to the formation of a hot S_1 state. The model then accounted for vibrational relaxation within S_1, inferred from the slight blue shift and narrowing observed for the S_1 C=C band, and for the ground state recovery. The results of the analysis suggested that vibrational relaxation in S_1 occurs within 450 fs, while the $S_1 \rightarrow S_0$ decay occurs in about 9 ps. In a further study [34] the same authors managed to observe also Raman features pertaining to the S_2 state, which appear as very intense and broad bands peaked at about 1100 cm^{-1} (methyl rocking), 1313 cm^{-1}(C-C stretching) and 1655 cm^{-1} (C=C stretching). These features rapidly decay in less than 200 fs, signaling the transition towards the S_1 state. The high frequency band initially peaked at 1655 cm^{-1} decays in intensity and is replaced by a narrower blue shifted band peaked at 1760 cm^{-1}, previously assigned to the C = C stretching absorption in S_1. The authors excluded the involvement of dark states in the $S_2 \rightarrow S_1$ internal conversion because they observed a smooth transition between the signals assigned to S_2 and those assigned to S_1.

More recently, we extended the analysis of carotenoids using FSRS, by studying the excited state relaxation dynamics of a carbonyl carotenoid, trans-β-apo-8'-carotenal (shortly indicated as apocarotenal) [18,19]. As already mentioned in the introduction, the presence of an asymmetric C=O substituent on the polyene chain confers peculiar properties to carotenals, the most relevant being a significant shortening of

their excited state lifetime in polar solvents, that has been attributed to the formation of ICT states. Initial FSRS studies on apocarotenal were conducted dissolving the molecule in cyclohexane, as to exclude the complications due to the presence of charge transfer states. The aim of these studies was to deeply analyze the excited state dynamics and in particular the internal vibrational redistribution (IVR) processes occurring in S_1. The SRS spectrum of apocarotenal in cyclohexane is quite similar to that of β-carotene, with the C=C stretching mode absorbing at 1528 cm^{-1} in the ground state. The sample was excited using an actinic pulse at 400 nm and the spectra were measured in a time interval up to 20 ps. The FSRS spectra measured at different delays upon actinic excitation are reported in Fig 3.

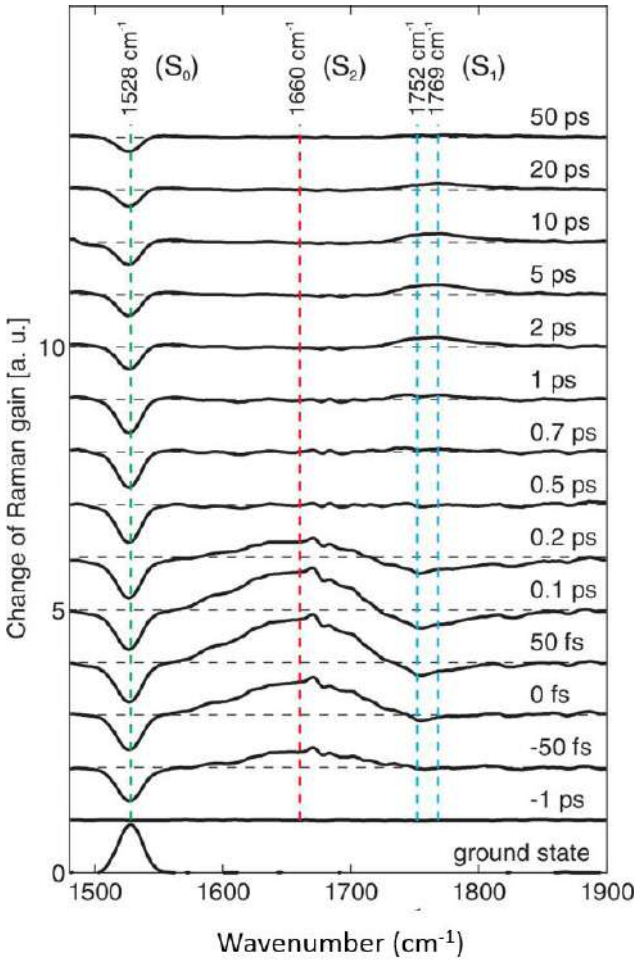


Fig 3. FSRS spectra measured at increasing delay times for apocarotenal in cyclohexane. The ground sate spectrum is reported at the bottom. Reprinted from https://doi.org/10.1063/1.4879060 with permission of AIP publishing.

As shown in Fig 3, the spectra measured at short delays present a very broad positive band peaked at about 1660 cm^{-1}, that, in analogy with what observed for β-carotene, is assigned to the upshifted C=C absorption in the S_2 state. Furthermore, a negative band peaked at 1528 cm^{-1} is observed, attributed to the bleaching of the ground state C=C absorption. The broad band assigned to S_2 decays very quickly, and is replaced on a timescale >1ps by a narrower peak located at 1752-1769 cm^{-1}, assigned to the C=C absorption

in S_1. A close observation of the spectral evolution in the 50 fs to 1 ps time interval evidences a peculiar phenomenon. The S_1 C=C band does not rise immediately upon the decay of the broad signal attributed to S_2: the band at about 1760 cm^{-1} initially presents a negative sign, then goes to zero and becomes positive in about 1 ps. This sign inversion was attributed to fast vibrational relaxation in the S_1 state. The very fast $S_2 \rightarrow S_1$ internal conversion indeed populates high vibrational levels of the S_1 state. The vibrational population subsequently relaxes undergoing a population inversion from a higher vibrational level towards the fundamental vibrational level of S_1. A theoretical model was proposed and applied to fit the dynamics of the signal at 1760 cm^{-1}. The model considered three vibrational levels within the S_1 excited state vibrational manifold, separated by an energy gap, which slightly decreases with the increasing vibrational energy of the levels. A series of equations were implemented to describe the quantum mechanical relaxation of the system, as detailed in reference [18]. The model allowed to fit the kinetic trace at 1760 cm^{-1} with high accuracy and to retrieve the rate constants associated with the decay of the different vibrational levels of S_1. The values obtained for the lifetimes were around 300 fs for the two higher vibrational levels and 600 fs for the lowest energy level, thus demonstrating that IVR in S_1 is complete in about 1 ps. The excited state relaxation then proceeds with the recovery of the ground state, occurring in about 30 ps in cyclohexane.

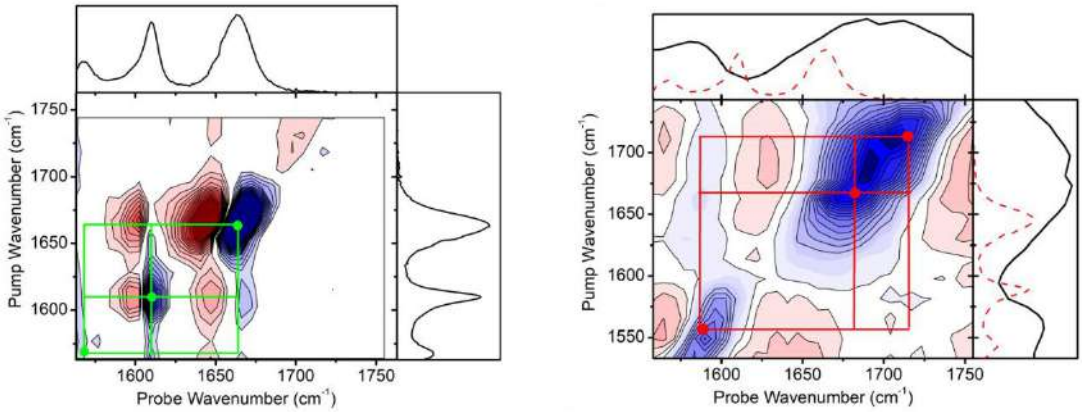


Fig 4. (A) Ground state 2D-IR spectrum of 8-apocarotenal in chloroform, registered with an IR-pump-IR-probe delay of 500 fs. (B) Corresponding excited state spectrum registered 3 ps after the excitation with a 400 nm pulse, where the two peaks attributed to S_1 and ICT states at 1685 cm^{-1} and 1715 cm^{-1} are highlighted with a red dot. Reprinted with permission from https://doi.org/10.1021/jp505473j. Copyright 2014 American Chemical Society.

Further studies on apocarotenal have been performed using time resolved infrared techniques, in particular Vis-pump/MidIR-probe spectroscopy (TRIR) [5,11] and transient 2-dimensional infrared spectroscopy (T-2DIR) [19]. These studies have been principally devoted on clarifying the role of the ICT state in this carbonyl carotenoid and the mechanism and dynamics of its formation. Indeed, by measuring TRIR and visible pump-probe spectra in different solvents, it has been established that the polarity of the external medium exerts a notable influence on the excited state lifetime of this molecule. Furthermore, an evolution of the S_1 C=C band was found to occur on the 2-4 ps timescale, which was interpreted as arising from the formation of the ICT state. T-2DIR measurements were executed both in cyclohexane, where the ICT state does not contribute to the dynamics, and in chloroform, where this state is populated. The spectra measured in chloroform presented two peaks around 1700 cm^{-1}, which were assigned as the C=C band of the S_1 and ICT states respectively, as shown in Fig 4. The band assigned to the ICT state was found to rise with a slower kinetics as compared to that of S_1. A theoretical analysis was furthermore performed, suggesting that population of the ICT state is promoted by a structural distortion of the polyene chain, which localizes a larger amount of negative charge on the C=O moiety. Concerning the nature of the ICT state, it was proposed

that it results from a repopulation of the $1B^{+}_{u}$ state, whose energy decreases below that of the $2A^{-}_{g}$ state as a result of this structural variation [5].

In a following paper, we analyzed the excited state dynamics of peridinin, a carbonyl carotenoid contained in the Peridinin-Chlorophyll protein (PCP) of photosynthetic organisms [32]. Peridinin is a highly substituted carotenoid with a C_{37} carbon chain (see Fig 1 for the structure). Its structure presents two carbonyl groups and a lactone ring, that highly influence the photodynamics of the molecule, inducing significant deviation from an idealized C_{2h} symmetry. The excited state relaxation mechanism of peridinin has been deeply investigated [4,36-38]. Because of the presence of different substituents, the relaxation dynamics of peridinin strongly depends on the solvent polarity. This behavior has been attributed to the possibility of populating an excited state with charge transfer character. The Raman spectra registered for peridinin in its electronic ground state in cyclohexane and chloroform are reported in Fig 5.

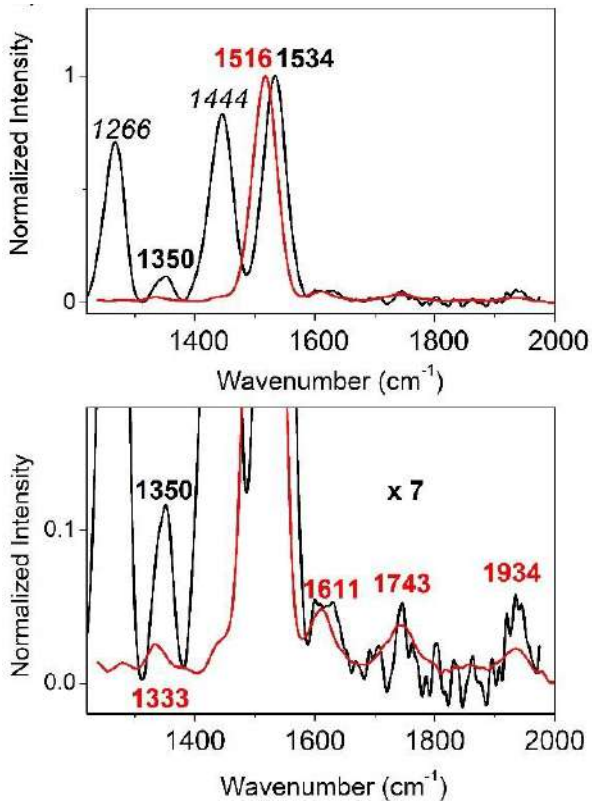


Fig 5. Stimulated Raman spectra of Peridinin in cyclohexane (black line) and chloroform (red line). The high wavenumber region is highlighted in the bottom panel. The band at 1444 cm^{-1} is assigned to cyclohexane. Reprinted from https://doi.org/10.1063/1.4915072 with permission of AIP publishing.

In cyclohexane, the spectrum presents an intense band peaked at 1534 cm^{-1} (1516 cm^{-1} in chloroform) assigned to the C=C stretching and a less intense peak at 1350 cm^{-1} (barely visible in chloroform) due to the C-C stretching. In the high wavenumber region, small signals are observed at 1611, 1743 and 1934 cm^{-1}. The assignment of these bands is less robust, most of the previous works attribute them to absorption from the carbonyls and lactone substituents [35]. Although the absorption of carbonyls is in principle not expected in the Raman spectrum, nevertheless it is generally accepted that the conjugation with the polyene chain can promote some Raman activity of these modes.

In order to gain insights about the shift of the Raman modes in the excited states of peridinin in cyclohexane upon excitation at 400 nm, and with a delay of 10 ps. The obtained spectrum is reported in Fig 6, together with the ground state SRS of the sample.

The spectrum presents two negative peaks at 1534 and 1350 cm^{-1} corresponding to ground state bleachings, and two small positive peaks at 1700 and 1760 cm^{-1}. Comparison with the transient infrared spectrum measured for the same molecule [35], allowed to assign the peak at 1700 cm^{-1} to the upshifted C=C stretching in the S_1 state and the band peaked at 1760 cm^{-1} to the lactone C=O. Measurements were tentatively repeated in chloroform, but the intensity of the acquired signals resulted too low compared to the noise. On the other hand, also for this molecule, the acquisition of TRIR and T-2DIR spectra in both cyclohexane and chloroform allowed to clearly identify the signatures of the ICT state in the more polar solvent.

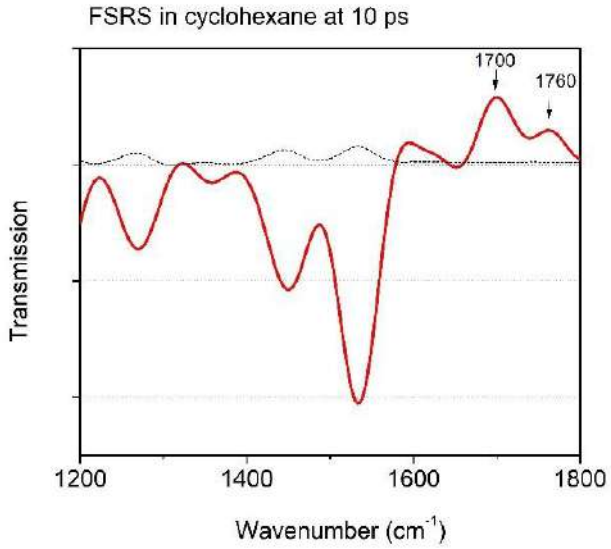


Fig 6. FSRS spectrum of peridinin in cyclohexane, measured with a delay of 10 ps after actinic excitation at 400 nm (red line). The SRS of the sample in the ground state is reported at the bottom as a black dotted line. Reprinted from https://doi.org/10.1063/1.4915072 with permission of AIP publishing.

The analysis of the excited state relaxation of biologically relevant carotenoids with FSRS has been furthermore extended to fucoxanthin, a carotenoid contained in several photosynthetic proteins [39]. The authors in this case varied the wavelength of the actinic pulse so as to favor the formation of the ICT state. By registering the FSRS spectra with two different excitation wavelengths, they were able to identify Raman bands pertaining to the ICT state. In particular, it was found that the C=C stretching, which absorbs at 1530 cm^{-1} in the ground state, is upshifted to 1735 cm^{-1} in the S_1 state and to 1550 cm^{-1} in the ICT state. The authors also observed that an equilibrium exists between the S_1 and ICT states that are coupled through the mode at 1550 cm^{-1}.

Finally, in a recent study, FSRS was applied to the analysis of the excited state dynamics of carotenoids contained in the photosynthetic antenna complex LHCII [40]. The analysis of FSRS spectra allowed to evidence the different dynamics of the two xanthophylls contained in this protein, namely lutein and neoxanthyn. A detailed analysis of the FSRS spectra, recorded with different actinic excitation wavelengths, such as to be selective for the different carotenoids, allowed to disentangle the different roles played by lutein and neoxanthin in the protein. It was found that one of the two luteins has the function

of transferring energy to the chlorophyll *a* molecules also present in the protein. Indeed, its excited state lifetime results much shorter if compared with that measured for luteins in solution, because of the energy transfer channel. The other lutein and the neoxanthin are not involved in energy transfer to chlorophylls, and upon light absorption undergo excited state decay with lifetimes similar to those observed in solution. The different contributions were extracted thanks to a detailed kinetic analysis of the FSRS spectra recorded upon excitation at different wavelengths, and in particular through the analysis of the kinetics of the excited state C=C band, which is found at slightly different frequencies for the different carotenoids.

4 Conclusion

Femtosecond stimulated Raman spectroscopy is an extremely suitable technique for studying the excited state relaxation dynamics of carotenoids, which present distinctive Raman bands in both the ground and excited states. The significant time and spectral resolution of this technique allows to clearly follow the dynamics of very fast phenomena, such as the internal conversion from the S_2 to the S_1 state, and the vibrational relaxation of the initially hot S_1 states. The measurements described in this review demonstrate that in most cases internal vibrational relaxation occurs in carotenoids on a fast timescale of about 500 fs. Other issues concerning the photodynamics of carotenoids have also been addressed such as the possibility that intermediate dark states could be involved. It has been found that in most of the analyzed systems the presence of dark states can be excluded, based on the analysis of the FSRS kinetics. Finally, in case of carbonyl carotenoids it has been found that combining time resolved Raman and IR measurements allows to obtain important information concerning the presence of an ICT state and to analyze both the dynamics of its formation and its role in the relaxation of the molecule.

Acknowledgements

The authors acknowledge support from the European Union Horizon 2020 research and innovation program under grant agreement n. 871124 Laserlab-Europe

References

1. Enriquez M M, Fuciman M, LaFountain A M, Wagner N L, Birge R R, Frank H A, The Intramolecular Charge Transfer State in Carbonyl-Containing Polyenes and Carotenoids. *J Phys Chem B*, 114(2010)12416-12426.
2. Gradinaru C C, Kennis J T M, Papagiannakis E, van Stokkum I H M, Cogdell R J, Fleming G R, Niederman R A, van Grondelle R, 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*, 98(2001)2364–2369.
3. Knecht S, Marian C M, Kongsted J, Mennucci B, On the Photophysics of Carotenoids: A Multireference DFT Study of Peridinin. *J Phys Chem B* 117(2013)13808–13815.
4. Polivka T, Sundstrom V, Ultrafast Dynamics of Carotenoid Excited States. From Solution to Natural and Artificial Systems. *Chem Rev*, 104(2004)2021–2072.
5. Donato M D, Centellas M S, Lapini A, Lima M, Avila F, Santoro F, Cappelli C, Righini R, Combination of Transient 2D-IR Experiments and Ab Initio Computations Sheds Light on the Formation of the Charge-Transfer State in Photoexcited Carbonyl Carotenoids, *J Phys Chem B*, 118(2014)9613–9630.
6. Balevičius V(Jr), Lincoln C N, Viola D, Cerullo G, Hauer J, Abramavicius D, Effects of tunable excitation in carotenoids explained by the vibrational energy relaxation approach, *Photosynth Res*, 135(2018)55-64.
7. Tavan P, Schulten K, Electronic excitations in finite and infinite polyenes, *Phys Rev B*, 36(1987)4337–4358.
8. Gacek D A, Holleboom C-P, Liao P-N, Negretti M, Croce R, Walla P J, Carotenoid dark state to chlorophyll energy transfer in isolated light-harvesting complexes CP24 and CP29, *Photosynth Res*, 143(2020)19–30.
9. Beck W F, Bishop M B, Roscioli J D, Ghosh S, Frank H A, Excited state conformational dynamics in carotenoids: Dark intermediates and excitation energy transfer. *Arch Biochem Biophys*, 572(2015)175–183.
10. Hashimoto H, Yanagi K, Yoshizawa M, DarioPolli, Cerullo G, Lanzani G, Silvestri S D, Gardiner A T, Cogdell R J, The very early events following photoexcitation of carotenoids, *Arch. Biochem Biophys*, 430(2004)61–69.

11. Ragnoni E, Donato M D, Iagatti A, Lapini A, Righini R, Mechanism of the Intramolecular Charge Transfer State Formation in all-trans-β-Apo-8′-carotenal: Influence of Solvent Polarity and Polarizability, *J Phys Chem B*,119(2015)420–432.
12. Ehlers F, Wild D A, Lenzer T, Oum K, Investigation of the S_1/ICT S_0 Internal Conversion Lifetime of 4'-apo-β -caroten-4'-al and 8'-apo-β-caroten-8'-al: Dependence on Conjugation Length and Solvent Polarity, *J Phys Chem A*, 111(2007)2257–2265.
13. Kopczynski M, Ehlers F, Lenzer T, Oum K, Evidence for an Intramolecular Charge Transfer State in 12'-Apo-β-caroten-12'-al and 8'-Apo-β-caroten-8'-al: Influence of Solvent Polarity and Temperature, *J Phys Chem A*, 111(2007)5370–5381.
14. Zigmantas D, Hiller R G, Yartsev A, Sundström V, Polívka T, Dynamics of Excited States of the Carotenoid Peridinin in Polar Solvents: Dependence on Excitation Wavelength, Viscosity, and Temperature, *J Phys Chem B*, 107(2003)5339–5348.
16. Polivka T, Kaligotla S, Chábera P, Frank H A, An intramolecular charge transfer state of carbonyl carotenoids: implications for excited state dynamics of apo-carotenals and retinal, *Phys Chem Chem Phys*, 13(2011)10787–10796.
17. Kosumi D, Kajikawa T, Okumura S, Sugisaki M, Sakaguchi K, Katsumura S, Hashimoto H, Elucidation and Control of an Intramolecular Charge Transfer Property of Fucoxanthin by a Modification of Its Polyene Chain Length, *J Phys Chem Lett*, 5(2014)792–797.
18. Kardaś T M, Ratajska-Gadomska B, Lapini A, Ragnoni E, Righini R, Donato M D, Foggi P, Gadomski W, Dynamics of the time-resolved stimulated Raman scattering spectrum in presence of transient vibronic inversion of population on the example of optically excited trans-β-apo-8′-carotenal, *J Chem Phys*, 140(2014)204312; doi.org/10.1063/1.4879060.
19. Donato M D, Ragnoni E, Lapini A, Kardaś T M, Ratajska-Gadomska B, Foggi P, Righin R, Identification of the Excited-State C═C and C═O Modes of trans-β-Apo-8′-carotenal with Transient 2D-IR-EXSY and Femtosecond Stimulated Raman Spectroscopy. *J Phys Chem Lett*, 6(2015)1592–1598.
20. Siebert T, Engel V, Materny A, Kiefer W, Schmitt M, 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(2003)8355–8362.
21. Siebert T, Maksimenka R, Materny A, Engel V, Kiefer W, Schmitt M, The role of specific normal modes during non-Born-Oppenheimer dynamics: the $S_1 \rightarrow S_0$ internal conversion of β-carotene interrogated on a femtosecond time-scale with coherent anti-Stokes Raman scattering, *J Raman Spectrosc*, 33(2002)844–854.
22. Siebert T, Schmitt M, Engel V, Materny A, Kiefer W, Population Dynamics in Vibrational Modes during Non-Born-Oppenheimer Processes: CARS Spectroscopy Used as a Mode-Selective Filter, *J Am Chem Soc*,124(2002) 6242-6243.
23. McCamant D W, Kim J E, Mathies R A, Vibrational Relaxation in β-Carotene Probed by Picosecond Stokes and Anti-Stokes Resonance Raman Spectroscopy, *J Phys Chem A*, 106(2002)6030–6038.
24. McCamant D W, Kukura P, Mathies R A, Femtosecond Time-Resolved Stimulated Raman Spectroscopy: Application to the Ultrafast Internal Conversion in β-Carotene, *J Phys Chem A*, 107(2003)8208–8214.
25. Yoshizawa M, Kurosawa M, Femtosecond time-resolved Raman spectroscopy using stimulated Raman scattering. *Phys Rev A*, 61(1999)013808; doi.org/10.1103/PhysRevA.61.013808.
26. Frontiera R R. Mathies R A, Femtosecond stimulated Raman spectroscopy, *Laser & Photonics Rev*, 5(2011)102–113.
27. Yoshizawa M, Hattori Y, Kobayashi T, Femtosecond time-resolved resonance Raman gain spectroscopy in polydiacetylene, *Phys Rev B*, 49(1994)13259–13262.
28. Yoshizawa M, Aoki H, Hashimoto H, Vibrational relaxation of the $2A_g^-$ excited state in all-trans-β-carotene obtained by femtosecond time-resolved Raman spectroscopy, *Phys Rev B*, 63(2001)180301; doi.org/10.1103/PhysRevB.63.180301.
29. Kukura P, McCamant D W, Mathies R A, Femtosecond Stimulated Raman Spectroscopy, *Annu Rev Phys Chem*, 58(2007)461–488.
30. Dietze D R. Mathies R A, Femtosecond Stimulated Raman Spectroscopy. *ChemPhysChem*, 17(2016)1224–1251.

31. Han F, Liu W, Fang C, Excited-state proton transfer of photoexcited pyranine in water observed by femtosecond stimulated Raman spectroscopy. *Chem Phys*, 422(2013)204–219.
32. Mallick B, Lakhsmanna A, Umapathy S, Ultrafast Raman loss spectroscopy (URLS): instrumentation and principle. *J Raman Spectrosc*, 42(2011)1883–1890.
33. Noguchi T, Hayashi H, Tasumi M, Atkinson G H, Solvent effects on the carbon-carbon double bond stretching mode in the $1A_g^-$ excited state of β-carotene and two derivatives: picosecond time-resolved resonance Raman spectroscopy, *J Phys Chem*, 95(1991)3167–3172.
34. Kukura P, McCamant D W, Mathies R A, Femtosecond Time-Resolved Stimulated Raman Spectroscopy of the S_2 ($^1B_u^+$) Excited State of β-Carotene. *J Phys Chem A*, 108(2004)5921–5925.
35. Donato, M D, Ragnoni E, Lapini A, Foggi P, Hiller R G, Righini R, Femtosecond transient infrared and stimulated Raman spectroscopy shed light on the relaxation mechanisms of photo-excited peridinin, *J Chem Phys*, 142(2015)212409; doi.org/10.1063/1.4915072.
36. Bonetti C, Alexandre M T A, van Stokkum I H M, Hiller R G, Groot M L, van Grondellea R, and John T M. Kennis J T, Identification of excited-state energy transfer and relaxation pathways in the peridinin-chlorophyll complex: an ultrafast mid-infrared study, *Phys Chem Chem Phys*, 12(2010)9256–9266.
37. Frank H A, Cogdell R J, Carotenoids in Photosynthesis. *Photochem Photobiol*, 63(1996)257–264.
38. Papagiannakis E, van Stokkum I H M, Vengris M, Cogdell R J, van Grondelle R, Larsen D S, Excited-State Dynamics of Carotenoids in Light-Harvesting Complexes. 1. Exploring the Relationship between the S_1 and S* States, *J Phys Chem B*, 110(2006)5727–5736.
39. Redeckas K, Voiciuk V, Vengris M, Investigation of the S1/ICT equilibrium in fucoxanthin by ultrafast pump–dump–probe and femtosecond stimulated Raman scattering spectroscopy, *Photosynt Res*, 128(2016)169–181.
40. Vivancos J M A, van Stokkum I H M, Saccon F, Hontani Y, Kloz M, Ruban A, van Grondelle R, Kennis J T M, Unraveling the Excited-State Dynamics and Light-Harvesting Functions of Xanthophylls in Light-Harvesting Complex II Using Femtosecond Stimulated Raman Spectroscopy, *J Am Chem Soc*, 142(2020)17346–17355.

[*Received*: 24.11.2021; *accepted*: 15.12.2021]

Elena Ragnoni

Elena Ragnoni obtained her Ph D at LENS (European Laboratory for non Linear Spectroscopy) in atomic and molecular spectroscopy in 2014. In her thesis, she applied ultrafast time resolved Raman and Infrared spectroscopy to study the photophysics of carotenoids in solution and in protein environments. She works for a pharmaceutical company.

Tomasz Michał Kardaś

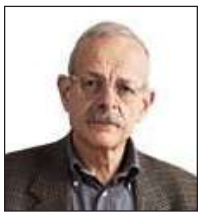
Tomasz Michał Kardaś is a co-founder and CSO of Fluence sp. z o.o. and a creator of Hussar – a nonlinear pulse propagation software. After receiving his Ph D (University of Warsaw in 2015) he worked at the University of Warsaw and Institute of Physical Chemistry PAS. His interest includes ultrafast nonlinear optics and laser physics.

Andrea Lapini

Andrea Lapini is tenure track Assistant Professor at the University of Parma, Italy. After obtaining his Ph D at LENS (European Laboratory for non Linear Spectroscopy), he worked as Researcher at LENS and INRIM (Italian Institute of Metrology). His research interests concern with the application of non-linear spectroscopy techniques to study the photo-dynamics of complex molecular systems and pure liquids.

Paolo Foggi

Paolo Foggi is full professor at the University of Perugia, Italy. His research concerns with the application of linear and non-linear spectroscopy to study the photophysics of molecular systems in solution and the dynamics of artificial membranes.



Roberto Righini

Roberto Righini is Professor Emeritus at the University of Florence, Italy. His research interests concern with the study of the structure and dynamics of molecular liquids, molecular photophysics, and applications of ultrafast spectroscopy.

Mariangela Di Donato

Mariangela Di Donato is Researcher at ICCOM-CNR in Florence, Italy. She previously worked at the free University Amsterdam, The Netherlands and the University of Florence, Italy. Her research focuses on the application of non-linear spectroscopy techniques to study the dynamics of photoinduced electron transfer in complex molecular systems and the photophysics of molecular photoswitches and triplet photosensitizers.