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Long-lived coherence in carotenoids

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Abstract. We use two-colour vibronic coherence spectroscopy to observe long-lived vibrational coherences in the ground electronic state of carotenoid molecules, with decoherence times in excess of 1 ps. Lycopene and spheroidene were studied isolated in solution, and within the LH2 light-harvesting complex extracted from purple bacteria. The vibrational coherence time is shown to increase significantly for the carotenoid in the complex, providing further support to previous assertions that long-lived electronic coherences in light-harvesting complexes are facilitated by in-phase motion of the chromophores and surrounding proteins. Using this technique, we are also able to follow the evolution of excited state coherences and find that for carotenoids in the light-harvesting complex the $|S_2\rangle|S_0\rangle$ superposition remains coherent for more than 70 fs. In addition to the implications of this long electronic decoherence time, the extended coherence allows us to observe the evolution of the excited state wavepacket. These experiments reveal an enhancement of the vibronic coupling to the first vibrational level of the C–C stretching mode and/or methyl-rocking mode in the ground electronic state 70 fs after the initial excitation. These observations open the door to future experiments and modelling that may be able to resolve the relaxation dynamics of carotenoids in solution and in natural light-harvesting systems.

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1. Introduction

Quantum coherence is an intrinsic feature of any quantum system, and it is important to identify instances in which it plays a significant role in naturally occurring biomolecular processes. Experimental evidence accumulated over the past decade [1]–[4] suggests that coherent transfer processes make a significant contribution to the remarkably efficient energy transfer in naturally occurring light-harvesting systems. Recent experiments [5]–[7] have provided the first direct evidence of coherent coupling between chromophores in the reaction centre of light-harvesting complexes, with coherence times of hundreds of femtoseconds. Carotenoids, present within both antenna complexes and the reaction centre, form an important part of nature’s light-harvesting machinery. In this study, we examine long-lived coherence phenomena in these linear polyenes, both in solution and within LH2 light-harvesting complexes.

Carotenoids are present in all known photosynthetic organisms and have several essential functions. Firstly, as accessory pigments, they absorb light and transfer the energy to other parts of the antenna complexes and eventually to the reaction centre. Secondly, carotenoids play an important role in photoprotection, rapidly quenching triplet excited states of chlorophylls, preventing the formation of highly reactive and damaging oxygen in its singlet excited state. Thirdly, it has also been suggested that carotenoids play a role in regulating energy transfer in light-harvesting antenna complexes, providing a pathway for energy dissipation to avoid over-excitation of the photosynthetic system [8].

Despite their undoubted importance, much mystery still surrounds carotenoids and the mechanisms by which they carry out these important roles. Even isolated in solution, the energy relaxation pathways and mechanisms are still contentious, with several different possibilities proposed; indeed, even the excited state energy structure remains an unresolved issue (see e.g. [9, 10] for reviews).

Carotenoids exhibit a strong absorption band in the spectral region around 400–500 nm, corresponding to a transition from the ground state (S₀, with A^- symmetry) to the second excited singlet state (S₂, with B^+ symmetry). The lifetime of this state is very short, relaxing to the first excited singlet state (S₁, a second state with A^- symmetry) in less than 200 fs and effectively quenching fluorescence from the S₂ state. The transition from the ground state to S₁ is dipole-forbidden due to the symmetry of carotenoids, which explains why the strong absorption is to the S₂ state. The S₁ state typically has a lifetime of the order of picoseconds, with the relaxation occurring predominantly by internal conversion, and virtually no radiative relaxation.
The rapid relaxation of the $S_2$ state, as well as the appearance of an additional peak in transient pump–probe spectroscopy, has led to proposals for the presence of other excited states between $S_2$ and $S_1$. Assignments have been made to a first $B_u^-$ state and a third $A_g^-$ state [11, 12]. Other groups have been unwilling to assign a symmetry label to states identified by a range of different experiments, instead ascribing their anomalous signals to $S^*$, $S_x$, $S^+$ or $S^\ddagger$ [9, 10], [13]–[17]. Further complicating the issue is the rich vibrational spectrum, with other groups proposing a relaxation pathway from $S_2$ to $S_1$ via a conical intersection. With this level of complexity and conjecture, it is clear that a new approach is needed [18]–[20].

Motivated by recent experimental evidence of long-lived electronic coherences in light-harvesting complexes [5]–[7], we have performed two-colour nonlinear experiments to identify specific coherent coupling between excited electronic and vibrational states in carotenoids. It has been suggested that the long-lived electronic coherences observed in light-harvesting complexes persist due to the protection provided by the surrounding protein matrix [5]–[7]. In the experiments discussed here, we are able to measure not only electronic coherences, but also vibrational coherences, and to identify any coupling within or between the electronic and vibrational states. In order to assess the role of the protein matrix in preserving these coherences, we have studied lycopene and spheroidene, both in solution and in the case of spheroidene in its natural environment within the LH2 light-harvesting complexes of the purple bacteria *Rhodobacter sphaeroides* 2.4.1 (Rb. sph.). The reason for studying these two carotenoids in particular is that the extensive body of research available provides a strong background and means for comparison, particularly for LH2 of *Rb. sph.*, which is one of the most extensively studied light-harvesting systems.

Lycopene, an unbranched conjugated molecule with $n = 11$ conjugated double bonds, as depicted in figure 1, was extracted from tomatoes. Spheroidene, which is similar in structure to lycopene but with $n = 10$ conjugated double bonds, was isolated from *Rb. sph.* The LH2 light-harvesting complexes were extracted from the relevant purple bacteria and placed in a buffer solution with the detergent lauryl-dimethylamide oxide (LDAO) to ensure the integrity of the protein structures. The absorption spectra for spheroidene and lycopene in a solution of n-hexane and in their respective light-harvesting complexes are shown in figure 2. Table 1 shows the energy (and wavelength) of the peak corresponding to the $S_0 \rightarrow S_2$ bright transition. Also shown in table 1 are the literature values for the energy of $S_1$ in each case, and the vibrational
Figure 2. The absorption spectra for lycopene and spheroidene in solution of n-hexane and (b) the absorption spectra of LH2 from Rb. sph. 2.4.1 and Rs. mol., with the carotenoid peaks indicated. The peaks from the bacteriochlorophyl, $Q_x$, B800 and B850 are also shown and used as a check for the stability of the complex.

Table 1. Vibrational and electronic energies of generally accepted states of lycopene and spheroidene in solution and LH2. Some uncertainty surrounds the energies of $S_1$, with the values quoted somewhat dependent on the method used.

| Lycopene in n-hexane [26, 27] | 19 880 (503 nm) |
| Lycopene in LH2 [26, 27] | 18 870 (530 nm) |
| Spheroidene in n-hexane [28, 29] | 20 700 (483 nm) |
| Spheroidene in LH2 [28, 29] | 19 570 (510 nm) |

energies in the electronic ground state for the three strongest vibrational modes (specifically, $C=C$ stretch, $C\equiv C$ stretch and a methyl-rocking mode). The vibrational energies change little between the different carotenoids and different environments, with each peak varying by less than 10 cm$^{-1}$ in the different systems and environments. The energy of the electronic transitions is, however, more dependent on the conjugation length and environment, as seen by the significant shifts. This information is used in determining excitation conditions for the experiments discussed below.

2. Experimental

Photon echo, four-wave mixing and photon echo peak shift are all third-order nonlinear spectroscopic techniques that are used to identify coherence and population dynamics in condensed matter systems [21]–[24]. The two-colour vibronic coherence spectroscopy [6, 21]
adopted here is a variation of these techniques that offers unprecedented selectivity in the interaction with molecular energy levels, and control over the formation of quantum coherences. In these two-colour vibronic coherence experiments, a standard three-pulse four-wave mixing configuration is used [21], where the excitation is provided by two synchronously pumped optical parametric amplifiers (OPAs) (modified Spectra Physics OPAs). The output from one OPA, referred to as pulse-1, is incident on the sample with the wavevector \( k_1 \). The output from the other OPA is split into two pulses, referred to as pulse-2 and pulse-3, which are incident on the sample with wavevectors \( k_2 \) and \( k_3 \), respectively. The third-order signals in each of the phase-matched directions are spectrally resolved and detected on a CCD (Ames Photonics, GARRY3000SD). In this paper, however, we concentrate on the signal emitted in the direction given by \( k_4 = -k_1 + k_2 + k_3 \). In the absence of any relaxation, the emitted signal will be at an energy given by \( \bar{h} \omega_4 = \bar{h}(-\omega_1 + \omega_2 + \omega_3) \), where \( \bar{h} \omega_1 \neq \bar{h} \omega_2 = \bar{h} \omega_3 \) are the photon energies for pulse-1, pulse-2 and pulse-3, respectively. The result is that the signal of interest is both spatially and spectrally resolved from each of the incident laser pulses and any other signals. This allows very weak signals that might otherwise be hidden by laser scatter to be detected and spectrally resolved with relative ease. The temporal delay between each of the pulses is varied to access different quantum mechanical pathways and the dynamics of the processes. The definitions of the delays \( t_{12} \) and \( t_{23} \) are the delays between pulse-1 and pulse-2 (positive when pulse-2 precedes pulse-1) and between pulse-2 and pulse-3 (positive when pulse-2 precedes pulse-3), respectively. The exception to this is when the delays are either both positive or both negative and \( t_{23} \) is redefined as \( t'_{23} = t_{23} - t_{12} \).

In the experiments discussed here, unless otherwise detailed, pulse-1 is set to be resonant with the bright transition, \( S_0 \rightarrow S_2 \), as determined from the absorption spectrum. The energy of pulse-2 and pulse-3 was varied in order to find states that are coherently coupled, as identified by the presence of an extended signal on scanning the delay of the third pulse. In general, there are four possible pathways that can generate a signal in this excitation sequence. These are represented by the diagrams in figure 3. In the first pathway, (a), the first two pulses, which have different photon energies, create a coherent superposition of two distinct excited states. A third-order signal is emitted in the phase-matched direction, provided that the coherence persists until the third pulse arrives. Varying the delay of the third pulse then measures the dynamics of this coherence. The presence of dark states intermediate to \( S_2 \) and \( S_1 \) in carotenoids has been proposed previously, leading to claims that coupling between these states and the \( S_2 \) state has been observed [25]. A laser pulse resonant with a dark transition would not ordinarily interact with the sample to produce a coherent superposition. When the photo-active state is coupled to the dark state, however, the state properties are mixed. The nominally dark state then acquires a finite and measurable oscillator strength, allowing it to interact with the laser field, and the third-order signal is further enhanced by the laser interactions with the large dipole moment of the bright transition. This pathway is able to identify the presence of coupling between excited states, and provides a means of revealing otherwise dark states, if they exist.

In the second pathway, figure 3(b), the first pulse excites the superposition between the ground state and the \( 1B_u^* \) optically active state. The second pulse then returns the system to the ground electronic state, but to a different vibrational level, leaving the system in a coherent superposition of the two vibrational levels. A third-order signal is then emitted in the phase-matched direction provided that the coherence persists until the third pulse arrives. Both of these processes, which we will refer to as the ee’ and gg’ pathways, are similar to coherent Raman experiments and provide a signal only so long as the established coherence is preserved.
Figure 3. The diagrammatic representations, together with energy level diagrams, show the two main pathways for the given pulse combinations. The ee’ pathway (R1 and NR1) proceeds via an excited state coherence and the gg’ pathway (R2 and NR2) proceeds via a ground electronic state coherence. In both cases, the rephasing (R) and non-rephasing (NR) pathways are shown.

In addition to the pathways depicted in figures 3(a) and (b) (which correspond to the ‘rephasing’ signal) the case where the order of the first two pulses is reversed can also provide a signal for both the ee’ and gg’ coherences, as depicted in figures 3(c) and (d). This pulse ordering, referred to as the non-rephasing signal, is, however, less likely in the gg’ case as it requires an initial population in the higher vibrational level. Given vibrational energy spacings of more than 1000 cm$^{-1}$ and thermal energy at room temperature of 200 cm$^{-1}$, the upper vibrational levels are unlikely to be populated. (Note that in these experiments rephasing is not actually possible in either case; we will, however, continue to use this terminology.)

A laser system consisting of a Spectra-Physics regenerative amplifier pumping two OPAs was used to give pulses of two distinct wavelengths. Typical pulse parameters were: duration 100 fs, bandwidth 300 cm$^{-1}$, pulse energies 10 nJ and repetition rate 1 kHz. The samples under investigation were placed in a 1 mm quartz cuvette, with concentrations determined so as to have absorbance $a \approx 1$. The samples were stirred continuously to prevent damage and ensure that fresh molecules/complexes were in the interaction region for each shot of the laser. Absorption spectra of the sample were taken at regular intervals to ensure that the sample remained undamaged. For each experimental configuration, data of the pure solvent were also taken to assess the effects of any solvent interactions, allowing us to rule out contributions from such processes in our discussion and analysis of the results.

3. Results and discussion

3.1. Lycopene in n-hexane

With pulse-1 set to 19 880 cm$^{-1}$ (503 nm), resonant with the $S_0 \rightarrow S_2$ bright transition, the wavelength of pulse-2 and pulse-3 was varied from 506 to 538 nm. In most cases, no extended signal was observed, with only the non-resonant (pure four-wave mixing) signal present when
Figure 4. (a) The spectrally resolved third-order signal from lycopene in n-hexane. Pulse-1 has a wavelength of 503 nm and pulse-2 and pulse-3 have a wavelength of 532 nm. The signal extends out to a $t_{23}$ delay of nearly 2 ps, indicating coherent coupling between states. The modulations indicate interference with another state and/or transition. (b) The same data spectrally integrated, with the fit to an oscillating exponential decay in red, giving a coherence time of 1000 fs and a beat period corresponding to an energy splitting of 156 cm$^{-1}$.

all three pulses were temporally overlapped. When the energy of pulses 2 and 3 was set to $18\,800 \pm 150$ cm$^{-1}$ (532 $\pm$ 4 nm), however, a signal extending well beyond the pulse overlap region was observed, indicating the presence of a long-lived coherence. Figure 4(a) shows the spectrally resolved signal as a function of delay between pulse-2 and pulse-3 ($t_{23}$) obtained under these excitation conditions, and with the delay between pulse-1 and 2 ($t_{12}$) set to zero. Given an energy separation between pulse-1 and pulse-2 of 1080 cm$^{-1}$, corresponding to an oscillation period of 30 fs, the presence of signal out to $t_{23} = 2$ ps implies that the quantum superposition remains coherent for more than 60 oscillations of the phase. The beating observed in the data is not due to this oscillation in the phase, as the period does not match and there is no phase
stability between the first two pulses of different colour, so any such interference will not appear as beating regardless. The beating indicates the presence of another pathway involving quantum levels of similar energy.

Figure 4 shows the spectrally integrated signal as a function of \( t_{23} \). A good fit to these data is obtained by an oscillating exponential decay (red line) of the form
\[
I_{\text{sig}} = e^{-2t/T} \left[ A + B \sin(\omega t + \phi) \right],
\]
where \( T_2 = 1000 \text{ fs} \) corresponds to the decoherence time of the coherently coupled states, \( B/A = 0.9 \) is the modulation depth, and \( 2\pi/\omega = 214 \text{ fs} \) is the beat period, corresponding to an energy separation of 156 cm\(^{-1}\).

An important corollary of the long coherence time is that the superposition state must also have a lifetime of at least this duration. This virtually rules out the participation of the \( 1B_u^+ \) state, as its lifetime has previously been measured to be less than 200 fs. We therefore consider the \( \text{gg}' \) pathway and note that the energy difference between the two laser pulses is 1080 cm\(^{-1}\) (with a pulse bandwidth of 200 cm\(^{-1}\)), which is very close to the energy of the methyl-rocking mode and the C–C stretching mode in the ground electronic state. The lifetime of these states is much greater than for the \( S_2 \) state, which makes it much more probable that this \( \text{gg}' \)-type coherence is the origin of the long-lived signal.

Based on the same reasoning, the origin of the beats, which decay at the same rate as the total signal, must also be a ground state coherence. There are two likely sources of this coherence: an additional low-frequency vibrational mode in addition to the higher-energy mode discussed above, or a coherence between those two high-frequency modes. Given the high density of low-energy vibrational modes, it seems unlikely that a single mode could be excited to generate the quantum beating. The energy difference between the C–C stretching mode and the methyl-rocking mode is 138 cm\(^{-1}\), very close to the 156 cm\(^{-1}\) beat period observed in figure 4, suggesting that this is the likely mechanism. Furthermore, experiments with the energy difference between the pulses shifted so that only the C–C stretch or methyl-rocking mode is excited leads to an extended signal with no beating, confirming that the beating is not due to low-frequency modes. The energy difference between these two vibrational modes, then there are two possible mechanisms that may account for the observed oscillation. In the first of these, the two vibrational modes are assumed to be coupled within the carotenoid, leading to interference and the generation of quantum beats. In the second scheme, polarization beats may be formed if two vibrational modes are completely isolated, in which case they would generate signals simultaneously that interfere during propagation.

In order to distinguish between these two possibilities, we apply our recently developed phase retrieval algorithm [30] to the data in figure 4 to obtain a two-dimensional (2D) frequency correlation diagram, figure 5. In the present two-colour experiments, the signal is analogous to heteronuclear NMR [31, 32], where the off-diagonal terms are enhanced, and any additional coupling within the off-diagonals is identified. On the vertical axis, where normally we would have absorption energy, we instead have a pseudo-Raman energy. This corresponds to the Fourier transform of the data with respect to delay, \( t_{23} \), and is labelled \( \omega_T \). This is the energy difference between the transitions excited by pulse-1 and pulse-2, and any other states to which they are coupled. The diagonal marked on this plot then corresponds to the expected emission energy, which is determined by adding the energy of pulse-3 to the value on the vertical axis. The data in figure 5 reveal the main signal to be approximately on the diagonal at (17 720 cm\(^{-1}\), –1080 cm\(^{-1}\)), and an additional signal on the off-diagonal at (17 760 cm\(^{-1}\),
Figure 5. The intensity 2D correlation spectrum obtained from the data in figure 4, with the pure four-wave mixing peak removed to allow the remainder of the signal to be seen. The off-diagonal nature of the second peak indicates that the beating is quantum beating due to states that are coherently coupled.

\(-1236 \text{ cm}^{-1}\), shifted by the expected 156 cm\(^{-1}\) in the \(\omega_T\) direction only. The presence of the second peak on the off-diagonal indicates that the two coherent vibrational modes are indeed coherently coupled, and the system exists in a coherent superposition of these vibrational states.

These measurements therefore show the presence of two coupled coherent vibrational modes, which have a dephasing time of 1 ps. This type of coherent phonon was previously seen by several groups using different methods and in different carotenoids [33]–[35], with lifetimes around 1 ps being reported. In most of these experiments, however, they were able to resolve the presence of the coherence only by the presence of beating in the signal, and the corresponding Fourier power spectrum. Using this methodology it is impossible to determine whether the different vibrational modes are coherently coupled, as we have revealed here.

In the analysis of the long-lived signal, all the fitting and discussion is based on the signal for delays greater than \(~400\) fs. Prior to this time, the signal amplitude increases rapidly, and given pulse durations of 100 fs, it is clear that there is some contribution from another pathway in this early time period. The rapid decay of this component is consistent with the reported lifetime of the \(S_2\) state, and given our excitation conditions, we attribute it to excited state dynamics. From the pathways represented in figure 3, this suggests that there is some contribution from an ee' coherence, although off-resonant excitation of the ee population pathway is also possible, and further analysis is required to confirm this.

Figure 6 shows the integrated signal as a function of \(t_{12}\) and \(t_{23}\), where pulse-1 and pulse-3 are the pulses moved in time. In this figure, it is clear that the phase of the beats is dependent not only on \(t_{23}\), as discussed previously, but also on \(t_{12}\). This is somewhat inconsistent with the previous assertion that the beats are due to the superposition of the two vibrational modes in
Figure 6. (a) 2D map of the integrated signal as a function of the two different delay times. Pulse-1 has a wavelength of 503 nm and pulse-2 and -3 have a wavelength of 532 nm. The phase of the beats is dependent on $t_{12} + t_{23}$ (as shown by the dashed line), indicating that pulse-1 is responsible for establishing the beats. This is made clearer in (b), where the $t_{12}$ value corresponding to the signal maximum is plotted for every $t_{23}$ delay. The signal is only shown for $t_{23} > 100$ fs, because before this time the pure four-wave mixing peak arising from pulse overlap dominates the signal.

the ground electronic state, established when the second pulse returns the system from $S_2$ to $S_0$, implying that the delay of the first pulse should not affect the phase of the superposition/beats. The observation that it does affect the phase suggests that the evolution of the $\langle S_2|S_0 \rangle$ coherence affects the vibrational level into which the system is returned by pulse-2. This is even more apparent in the LH2 complex, and we discuss this in more detail after we present these results.

3.2. Spheroidene in n-hexane

With pulse-1 set to 20700 cm$^{-1}$ (483 nm), resonant with the $S_0 \rightarrow S_2$ bright transition, the wavelength of pulse-2 and pulse-3 was varied from 483 to 530 nm. In most cases, as for lycopene, no extended signal was observed. When the energy of pulse-2 and pulse-3 was set to $19\,740 \pm 150$ cm$^{-1}$ (506 ± 4 nm), and $19\,200 \pm 100$ cm$^{-1}$ (521 ± 2 nm), however, extended signals were observed. Figure 7 shows the spectrally resolved signal as a function of $t_{23}$ obtained under these excitation conditions, and with $t_{12} = 0$. It is clear that when $\lambda_{2/3} = 506$ nm an extended signal with beating is present, whereas when $\lambda_{2/3} = 521$ nm an extended signal is present, but without any beating. The energy difference between pulse-1 and pulse-2 and pulse-3 in these two cases is 960 and 1510 cm$^{-1}$, respectively, with the beat period in the former corresponding to an energy separation of 147 cm$^{-1}$. These energy differences correspond to the vibrational energies from table 1. The signal when $\lambda_{2/3} = 506$ nm is then due to the superposition of the coherent $\text{C} - \text{C}$ stretching and methyl-rocking modes, and the signal when
Figure 7. (a) The spectrally resolved two-colour electronic coherence data for spheroidene in n-hexane, with $\lambda_1 = 473$ nm and $\lambda_{2,3} = 506$ nm. Similar to the data for lycopene, an extended signal is observed with quantum beating, indicating the presence of long-lived coherent coupling between vibrational levels in the ground electronic state. (b) With $\lambda_{2,3} = 521$ nm an extended signal is present, but with shorter lifetime and no beating. (In both cases, the data have been scaled so the extended signal can be seen more clearly, meaning that the signal close to zero delay appears saturated.)

$\lambda_{2/3} = 521$ nm is due to the formation of a coherent C=C stretching vibrational mode. The lifetimes of these vibrational coherences were measured to be 600 and 250 fs, respectively. The value for the C—C stretch and methyl-rocking modes is significantly shorter than that for lycopene. This is comparable to previous results [34] that show a decrease in the dephasing time of the methyl-rocking mode, but a slight increase in the dephasing time of the C—C stretching mode, as the number of conjugated double bonds goes from 11 to 9 in $\beta$-carotene homologues. The coherence time of the C=C stretching mode is shorter still, in agreement with [34], and can be explained by the higher energy and hence higher frequency of vibration, meaning that small fluctuations will lead to greater dephasing.

### 3.3. Spheroidene in LH2

In the LH2 light-harvesting complex, carotenoids are held in place by a protein matrix, all of which is in solution. The protein matrix changes the environment of the carotenoid molecules, which leads to a red-shift of the bright transition, as identified by the absorption spectra in figure 2. The vibrational energy levels, however, remain virtually unchanged, suggesting that the carotenoids remain in their all-trans isomer [29] (in contrast to reaction centres where the carotenoids adopt the 15-cis formation [29]). Another effect of the protein matrix is the protection of electronic coherences between states localized on different molecules, as evidenced in the reaction centre [6]. To date, no such enhancement of coherences involving carotenoids has been observed.

Figure 8(a) shows the spectrally resolved signal as a function of $t_{23}$ for spheroidene in LH2, with $t_{12} = 0$. In this case, the energy of pulse-1 was resonant with the bright transition (19 570 cm$^{-1}$ or 510 nm), and the energy of pulse-2 and pulse-3 was set to where an
Figure 8. (a) The spectrally resolved signal from spheroidene in LH2, with pulse-1 resonant with the bright transition and the other two resonant with the transition from S₂ to the excited vibrational levels in S₀. Quantum beats are again seen due to the superposition of the C—C stretching and methyl-rocking modes. A wavelength shift accompanies the beats as the system oscillates between the two different vibrational modes. (b) The spectrally integrated data and fit, together with that for spheroidene in hexane, revealing significant enhancement of the coherence time in LH2.

extended signal was seen (18 620 ± 100 cm⁻¹ or 537 ± 3 nm). These energy differences once again correspond to the vibrational energies of the C—C stretching/methyl-rocking modes of spheroidene. Under these conditions a long-lived signal is seen, with beating corresponding to the energy separation between the C—C stretching mode and the methyl-rocking mode of the carotenoid. In addition, the beats can be seen to shift in wavelength as t₂₃ is varied and the system oscillates between the two vibrational modes.

As described above, the integrated signals were calculated and fitted with an oscillating exponential decay to obtain the beat period and lifetime of the vibrational coherences, as shown in figure 8(b). In the case of spheroidene, the decoherence time was calculated to be 1080 fs (i.e. twice the decay constant of 540 fs). This value is nearly a factor of two greater than the value measured for spheroidene in n-hexane. This enhancement of the vibrational coherence time strongly supports the suggestion that the protein matrix protects the chromophores from the external environment, thereby increasing coherence times. Furthermore, these long vibrational coherence times and the enhancement within the light-harvesting complex further support the conclusions of [5]–[7] that the electronic coherences are maintained as a result of correlated
The integrated signal as a function of $t_{12}$ and $t_{23}$ shows a slightly extended signal in $t_{12}$ (in addition to the long-lived signal in $t_{23}$), indicating the enhancement of the $S_0 - S_2$ decoherence time. Away from $t_{23} = 0$, the signal peaks not at $t_{12} = 0$ but at $t_{12} = 7$ fs, providing details of the excited state evolution.

Further analysis of these data yields the plot in figure 9 showing the signal amplitude as a function of both $t_{12}$ and $t_{23}$. The most striking feature of this plot is that the signal present for long $t_{23}$ delays is peaked not at $t_{12} = 0$, as might be expected (and as is the case when $t_{23} = 0$), but rather at $t_{12} = -70$ fs (i.e. where pulse-1 arrives 70 fs before pulse-2). With 100 fs pulses it is clear that the peak at $t_{12} = -70$ fs occurs while there is still some pulse overlap, making detailed analysis difficult, and is highly susceptible to the effects of any pulse distortions such as chirp. The shape and location of the peak at $t_{12} = t_{23} = 0$, together with comparisons to the signal in just the solvent, however, strongly suggest that this response is from the sample. For the signal at $t_{23} = 0$, the maximum is at $t_{12} = 0$, where signal from the solvent as well as the sample contributes to the overall signal. At the maximum of the first and all subsequent beats, however, the signal is peaked at $t_{12} = -70$ fs. The enhancement of the signal at $t_{12} = -70$ fs is made evident by examining the ratio of the signal strength at $t_{23} = 200$ fs compared to that at $t_{23} = 0$ fs. For the signal at $t_{12} = 0$, the ratio is 0.1, and at $t_{12} = -70$ fs, the ratio is 0.7. This is a factor of 7 greater, strongly suggesting that this is an electronic effect from the sample, and not due to chirp or other artefacts in the laser pulse or solvent response.

The evolution in $t_{12}$ is more or less the same for all values of $t_{23}$ where a signal is generated. This suggests that the general pathway responsible for generating the signal remains the $gg'$ pathway, and the evolution in $t_{12}$ provides information about the evolution of the system in the $\langle S_2|S_0 \rangle$ coherence, before it is returned to the ground electronic state.

Specifically, the fact that the signal extends beyond the pulse overlap regime (for pulse-1 and pulse-2) provides clear evidence that the lifetime of the electronic coherence between $S_0$ and $S_2$ in the carotenoid is greatly enhanced in the LH2 complex. If this is indeed the case, this nuclear motion of both the molecules involved and the surrounding proteins within the light-harvesting complex.
type of room temperature electronic coherence suggests that the protein structure of the LH2 complex may be providing even greater protection than previously thought.

Furthermore, the fact that the signal peaks not at $t_{12} = 0$, but exhibits an almost oscillatory evolution to the peak at $-70$ fs, provides details of the evolution of the excited state wavepacket. Specifically, we speculate that the initial Franck–Condon excitation to $S_2$ is not the optimal nuclear orientation for subsequent relaxation to the higher vibrational levels of $S_0$. As the system evolves following the initial excitation, the vibronic couplings mediated by the Franck–Condon factors are maximized 70 fs after excitation. This evolution thereby tells us in great detail about the evolution of the excited state wavefunction following excitation to $S_2$. In order to access all the information, further analysis and modelling of the carotenoid excited states is required. One further complicating factor is that, in addition to maximizing the vibronic coupling, the system will also be dephasing, leading to a decay in the signal strength and possibly a maximum in the signal before the vibronic coupling is maximized.

This interpretation is consistent with observations that the phase of the signal varies with both $t_{12}$ and $t_{23}$ in both LH2 and in solution. The dependence of the phase of the beats on $t_{12}$ arises as a result of the changing vibronic coupling strength, leading to different relative contributions from each of the vibrational modes and hence a different phase of the quantum beats.

Hauer et al previously showed that it is possible to control the vibrational mode selected using coherent control techniques [33]. Here, we have shown a way to probe the natural evolution of a selectively excited electronic state by observing the evolution of the vibronic coupling to different ground state vibrational modes.

4. Conclusions

We have shown that ground state vibrational coherences can be generated in carotenoids with coherence times in excess of 1 ps in solution. When the carotenoids are confined within the LH2 light-harvesting complex, the coherence times of these vibrational modes are substantially increased. This adds further support to previous assertions that long-lived electronic coherences in light-harvesting complexes are facilitated by in-phase motion of the chromophores and surrounding proteins. We have also presented results that suggest the presence of an excited state electronic coherence that remains coherent for more than 70 fs in spheroidene in LH2. In addition to the implications of the long electronic coherence time, it is apparent that in this experimental configuration the evolution of this coherence may reveal details of the excited state wavepacket dynamics. The energy landscape, level assignments and relaxation dynamics remain areas in which no clear consensus has emerged in the study of carotenoids. The ability to interrogate these systems and to follow and control their excited state dynamics has the potential to resolve many of the remaining ambiguities of interpretation. If this is achieved, there is considerable promise that these methods may be extended to reveal the electron dynamics in a wider range of complex condensed matter systems.

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