Coherence in carotenoid-to-chlorophyll energy transfer

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The subtle details of the mechanism of energy flow from carotenoids to chlorophylls in biological light-harvesting complexes are still not fully understood, especially in the ultrafast regime. Here we focus on the antenna complex peridinin–chlorophyll a–protein (PCP), known for its remarkable efficiency of excitation energy transfer from carotenoids—peridinins—to chlorophylls. PCP solutions are studied by means of 2D electronic spectroscopy in different experimental conditions. Together with a global kinetic analysis and multiscale quantum chemical calculations, these data allow us to comprehensively address the contribution of the potential pathways of energy flow in PCP. These data support dominant energy transfer from peridinin S2 to chlorophyll Qy state via an ultrafast coherent mechanism. The coherent superposition of the two states is functional to drive population to the final acceptor state, adding an important piece of information in the quest for connections between coherent phenomena and biological functions.

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Dynamics of energy transport in light-harvesting complexes is traditionally attributed to the peculiar properties of tetrapyrrole compounds, mainly chlorophylls (Chls) and bacteriochlorophylls. A particular family of marine organisms of the dinoflagellates phylum has instead evolved light-harvesting machinery, mainly relying on carotenoids (Cars) as major harvesting pigments. Their external antenna, the peridinin–chlorophyll a–protein (PCP), also collects light in the blue-green spectral region of the visible spectrum, where solar radiation peaks below sea level, and where Chls do not absorb (Fig. 1). In fact, the early publication of PCP high-resolution X-ray structure revealed that this water-soluble protein has a pseudo-two-fold symmetry and binds eight peridinins (Pers) clustered around two Chl molecules. The detailed structural information, together with the remarkable efficiency for excitation energy transfer (EET) from Car donors to Chl acceptors (~90%)\(^2\)–5, have inspired intense research among the biophysical community\(^6\)–12. While the properties of Cars as leading actors in photoprotection\(^13\) and non-photochemical quenching\(^14\) have been the object of several in-depth investigations in the last decades\(^15\)–17, the mechanistic and dynamic details of the excitation energy flow from Cars to Chls are still not fully understood. One of the main difficulties is a limited knowledge of the electronic properties of Cars\(^18\)–21. PCP, with its high content of Cars, represents therefore an ideal test case. Moreover, the presence of Pers, peculiar Cars substituted with polar groups, may lead to intriguing features.

Traditionally, Cars’ electronic properties are described with a three-level model, including a ground state, \(S_0\) (\(1\text{A}_g\))-\(1\text{B}_u\)), and two low-lying singlet excited states, \(S_1\) (\(2\text{A}_g\)) and \(S_2\) (\(2\text{B}_u\)). It follows that \(S_0 \rightarrow S_1\) transition is symmetry-forbidden and that \(S_2\) is the first bright state\(^12\). Early femtosecond spectroscopy experiments on PCP\(^3\)–5 revealed that after photoexcitation, \(S_2\) state rapidly relaxes to \(S_1\) state via internal conversion in tens–hundreds of femtoseconds. These experiments led to models in which the excitation energy was mainly funneled from the \(S_1\) state of Per to the \(Q_x\) state of Chl with a characteristic time constant of 2.5–3.5 ps\(^8\).

However, the weak oscillator strength for the \(S_0 \rightarrow S_1\) transition and the remarkable efficiency of EET process toward Chl appeared immediately contradictory. Therefore, also considering the presence of polar groups in both the backbone and the terminal rings of Per, the formation of an intramolecular charge transfer (ICT) state has been proposed. The ICT state was described as intimately coupled to the dark \(S_1\) state, and crucial for its capability to enhance the \(S_0 \rightarrow S_1\) transition dipole moment, leading to a more efficient EET between Per and Chl\(^9\).

Like for other antenna complexes bearing Cars, femtosecond spectroscopic experiments on PCP have also suggested the presence of ultrafast channels of EET from \(S_2\) to the \(Q_2\) bands of Chls\(^7\)–9,23 (\(Q_x\) and/or vibronic states of \(Q_y\)), also with possible contributions of coherent pathways\(^24\)–25. However, clear and direct evidence is still lacking to identify the main pathways of energy flow from Pers to Chls, and their timescales, including the role of vibrational modes on the dynamics.

Starting from the existing body of preliminary knowledge already available and having in mind the questions still open about the mechanism of EET, the role of \(S_2\) state in EET process in PCP is investigated. We also exploit the higher spectral resolution and the higher dimensionality of two-dimensional electronic spectroscopy (2DES) to relieve the spectral congestion of the multi-chromophoric system of the protein complex. Guided by multiscale quantum chemical simulations, 2DES spectra are acquired in different experimental conditions, exploiting the spectral filtering action of the exciting bandwidth to turn on, or off, from time to time the contributions of Pers. This procedure, together with high-level data analysis methodologies, allows a clear identification of the multiple channels of energy flow from Per to Chl with an unprecedented level of detail. The associated timescales are characterized, and the identification of important mechanistic details, including the presence of coherent transfer and the potential involvement of vibrational modes of Per, is assessed.
effects of specific residues placed around the terminal rings (Supplementary Fig. 2 and 3). These induce a strong electric field (Fig. 2b) oriented along the main axis of the pigment, thus largely stabilizing the S2 state. Additional details on this analysis are reported in Supplementary Note 1.

Of even further interest, the S2 → Qy calculated transfer rates for the pair Per624–Chl602 show a nontrivial probability distribution, close to a lognormal distribution (Supplementary Fig. 5). This has a tremendous effect, particularly for the interpretation of the experimental data. Indeed, while in the familiar normal distribution models, energy transfer is described by a single exponential function with a time constant equal to the center of the distribution, here the time parameter must be represented by a probability density function, as suggested by other authors in similar systems26,27.

Population dynamics. With the aim of capturing the details of the fastest EET channel, the experimental conditions for the 2DES measurements have been set according to these theoretical predictions and, thus, the laser pulses have been tuned to the red edge of the linear absorption spectrum of Per, as shown in Fig. 1. Three different experiments were performed. In each, the exciting bandwidth was carefully modulated with pulse-shaping techniques to act as a spectral filter28–30 and turn off the contribution of the Per donor (laser 1), or progressively brought into resonance with Per (laser 2 and 3), to follow the effects of the coupling between Per and Chl.

The 2DES experiment with laser 1, detuned from the S2 absorption band, was taken as a control measurement to study the 2DES response of Chl in PCP. Absorptive 2DES maps recorded in this condition (Fig. 3a) reveal a behavior in full agreement with previous 2DES characterization of Chl solutions31–34. Indeed, the maps are dominated by an intense positive diagonal peak centered at 14 850 cm⁻¹ (position A in Fig. 3b), easily assigned to ground-state bleaching (GSB) and stimulated emission (SE) of the Qy state. The weaker cross-peak at (16 000, 14 850) cm⁻¹ (position B) can be attributed to the coupling of the Qy electronic transition with vibrational modes between 1000 and 1200 cm⁻¹, and to an ultrafast internal conversion process from Qy to Qx states, as detailed in previous works33,34.

2DES maps recorded at 100 fs with laser 2 (Fig. 3b) and 3 (Fig. 3c) reveal the presence of a strong negative feature at excitation energy > 17 000 cm⁻¹ (position C). This is due to excited-state absorption (ESA) signal from the Per S1 state that obscures, almost completely, the GSB- and SE-positive signals from S2 state. The rise of this strong signal in the ultrafast nonlinear response of PCP is widely documented in the literature3–5,8 and reflects the presence of the S2 → S1 internal conversion. In the experiments conducted with laser 3, together with the ESA from S1, a rapidly decaying ESA from S2 was also captured. Indeed, at early times (Fig. 3g), an additional negative feature is present at position D (17 500, 17 000) cm⁻¹. This feature disappears in <30 fs, in agreement with the short lifetime of S2. As discussed below, the dynamics of the main S1 ESA signal is strongly influenced not only by the rate of the S2 → S1 process but also by the presence of other ultrafast EET pathways affecting the population decay of S2. This implies that although the strong negative ESA features may hide other important contributions, they still retain important information on the competing transfer mechanisms that deplete the S2 state.

In fact, a detailed analysis of the signals at excitation frequencies > 17 000 cm⁻¹ under the three different exciting conditions unveiled important details on the Per → Chl EET. First, the trend shown by the three sets of 2DES measures disproves previous hypothesis that invoked the S2 → Qx pathway as responsible for the rise in the GSB signal in transient
Indeed, if $S_2 \rightarrow Q_x$ was the dominant pathway, the 2DES map would display a rising cross-peak at coordinates (17 500, 16 150) cm$^{-1}$, tentatively indicated with a dotted circle in Fig. 3a–c, but no rise at these coordinates was detected. Even assuming that this signal is canceled by the negative ESA contribution, the dynamics of ESA at these coordinates would have maintained some features of this pathway, detectable through kinetic models. This is not the case. First of all, no meaningful differences could be detected looking at the evolution

Fig. 3 Absorptive 2DES signal of PCP at 295 K. a–c Experimental and d–f simulated 2DES maps at a selected value of population time (100 fs) obtained with laser 1 (a, d), laser 2 (b, e), and laser 3 (c, f), respectively. More details of experimental and simulated data are reported in Supplementary Fig. 11–13. Maps are normalized to 1 at their maximum. The dots labeled from A to F in a pinpoint relevant positions as commented in the main text for all the laser bandwidths. g 2DES maps recorded with laser 3 at different population times
of the 2DES trace along t2 extracted, where the S2/Qy peak should show up and where only S1 → S0, ESA contribution is expected. Moreover, we verified that the decay of S2 population matches the rise of Qy (Supplementary Fig. 14). Indeed, we identified a clear rising signature at (17 500, 14 850) cm⁻¹ (position E in Fig. 3). The control measurement (laser 1) did not show this rise that, instead, became greater as we switched from laser 2 to laser 3 (Fig. 4a). The particular frequency coordinates, and the trend found among the three exciting conditions, suggest the attribution of this feature to an increasing signal due to S2 → Qy, EET.

At least two possible mechanisms of EET can be identified, as represented by the Feynman diagrams shown in Fig. 4. Together with the common incoherent population transfer pathway, a coherent mechanism can also be envisioned, where S2/Qy coherence prepared by the laser pulse after the second interaction dephases during the population time and gets localized on the Qy population (panel a). Assuming that this second coherent mechanism is active, we would also expect a concomitant rise of the signal at diagonal position A, witnessing an increase of the Qy population following the transfer from S2, as described by the kinetic schemes (Supplementary Methods 2).

The best fitting results were obtained from the kinetic model shown in Fig. 5a, where the time constants regulating the relaxation process are also highlighted. Following calculations, the rate of S2 → Qy process was described in terms of a lognormal distribution: 

$$p(t; \mu, \sigma) = \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{(\ln t - \ln \mu)^2}{2\sigma^2}\right);$$

with \(\ln \mu\) mean value and \(\sigma\) standard deviation of the distribution. This implies that, for the S2 → Qy process, a probability density function rather than a single time constant emerges from the fit. Applying this fitting model, we found \(\mu = 63\ \text{fs}\) and \(\sigma = 0.985\) (Fig. 5c). This process is competitive with the S2 → S1 internal conversion (≈150 fs estimated time constant). On a longer timescale, energy is transferred to Qy through the S1 → Qy channel (≈2 ps).

Figure 5b summarizes the time evolution of the states involved in EET, according to this fitting model. It should be noted that in the time and spectral window investigated, the population of the final acceptor Qy state, although fed by two distinct channels, derives mainly from S2.

Recently, another kinetic scheme has been proposed to describe EET from S2 to Qy, which relies on a preliminary step of relaxation of the S2 state geometry from the Franck-Condon to a distorted conformation. Our quantum chemical calculations, however, seem to rule out the presence of such a preliminary step as there is no trace of relaxed twisted configurations of Per in the S2 state.

As shown in Fig. 4b, specific information on the coherent regime of EET can be extracted from the dynamics of the diagonal peak, whose initial ultrafast rise has already been...
attributed to a purely coherent EET pathway. This rise could be fitted with an average time constant of 22 fs, which can be used to estimate the timescale in which coherent EET is relevant, as shown in Fig. 5c.

**Oscillating contributions.** The evolution of the 2DES maps along the population time, together with the population decays already described, revealed the presence of a lively beating behavior, mainly due to the activation of the strongly coupled vibrational modes of the Per. In particular, strong components beating at 1165, 1230, and 1565 cm\(^{-1}\) were detected. They matched the resonant Raman signals of Per in acetonitrile\(^{37}\) and the calculated spectral density of the Per (Fig. 2c and Supplementary Fig. 16).

The predominance of Per vibrational modes in the 2DES response, with respect to Chl modes, only just identifiable from noise, is expected in the light of the large Huang-Rhys factors characterizing these modes.

To check for the presence of electronic coherences between Per and Chl states, whose relevance has been recently suggested in the literature\(^{24,25}\), the beating behavior of the rephasing signal (recorded with laser 3) in the S₂/Q\(_y\) cross-peak region has been carefully scrutinized, applying a recently proposed method based on a time-frequency transform (TFT) analysis\(^{38,39}\). Given an oscillating trace, this analysis allows extracting not only the main frequency components contributing to the beating, as in conventional Fourier transform, but also their time behavior. The result is a bi-dimensional time-frequency plot, such as the one reported in Fig. 6d. Here the frequency and dephasing times of the relevant components are identified on the y- and x-axes, respectively. The TFT analysis of the 2DES trace at these positions (Fig. 6a) unveils four main oscillating contributions. The first three components have frequencies of approximately 1150, 1210, and 1550 cm\(^{-1}\) and dephasing times of several hundreds of femtoseconds. They correspond to the same vibrational modes of Per already identified in the analysis of the overall 2DES maps. The presence of Per vibrational coherences at these positions is in agreement with simulated data (Supplementary Fig. 16–19).

The fourth component is a quickly damped oscillation centered at \~1900 cm\(^{-1}\) (Fig. 6b–d), which dephases in \~20 fs. Its contribution was exhausted after 75 fs (panel c) and characterized by a large bandwidth, in agreement with the time-frequency indetermination principle. In the first tens of femtoseconds, this feature added to the longer-lived vibrational signals and produced negative amplitudes in Fig. 6d (Supplementary Fig. 9). More details on this investigation are reported in Supplementary Note 3. The same analysis performed at corresponding positions above diagonal produced similar results. Although the dominating contributions of the Per vibrational modes hinder the direct detection of the fourth broad component, its presence is indirectly manifested through negative amplitude features (Supplementary Fig. 10). The frequency and time behavior of this beating component, together with the peculiar coordinates at which it contributes, point toward the attribution of these dynamics to an electronic coherence between Per S₂ and Chl Q\(_y\).

In support of this interpretation, Fourier analysis (Fig. 6c) and time-frequency plots (Supplementary Fig. 7) generated from the traces at fixed emission frequency (colored dots in Fig. 6a) revealed that this ultrafast feature shifts accordingly to the excitation energy. Indeed, the sampling at increasing excitation energies results in the selection of Per with slightly higher S₂ site energies. As a consequence, the coherent superposition with Chl Q\(_y\) state would also beat at slightly higher frequencies.

The presence of the same beating frequency at the symmetrical position above the diagonal, expected for an electronic coherence\(^{40}\), could not be ascertained as this portion of 2DES maps is strongly dominated by vibrational modes of Per. The strong oscillations above diagonal are clearly visible also in Fig. 3b, c, g as a large cross-peak around (15 500, 17 000) cm\(^{-1}\) (position F in Fig. 3), strongly beating along the population time. The intensity of this contribution increased progressively with respect to the amplitude of the oscillations below diagonal, moving from laser 1 to laser 2 and 3, i.e., increasing the contribution of resonant S₂ states. These coherent signals have the frequency and the time behavior of Per vibrational coherences. However, they occur in a spectral region not predicted by the displaced harmonic oscillator model\(^{40}\) for these modes, suggesting the presence of more complex mechanisms. It has been recently suggested that vibrational modes of accessory pigments could work as a sink, absorbing the excess energy released by energy transfer due to the mismatch of donor and acceptor levels\(^{41,42}\). In the case of PCP, the strongly beating signal in the upper-left portion of the maps would represent the deposition into the Per vibrational modes of the excess energy released after the S₂ \(\rightarrow\) Q\(_y\) transfer. Further investigations are needed before definitive conclusions can be reached.

**2DES simulations.** All the experimental results described above can be framed in a unified picture with the help of theoretical simulations. Simulations of the 2DES signal are performed following the standard approach based on the third-order perturbation theory\(^{43,44}\), where a response function is fully convoluted with all the exciting pulses\(^{45}\). The simulation of the third-order optical response was fed with parameters obtained with the
quantum chemical calculations described above; here, however, only the excited states Q_y of Chl602 and S_2 of Per624 were considered for the sake of simplicity. Calculations indeed suggested that this pair provides the major contribution to the faster EET channel captured by the experimental data and therefore this choice allows simplification of the calculations without losing in generality.

Inhomogeneous broadening has been included, averaging over 200 realizations, and the real spectral profile of the exciting pulses has also been considered. Examples of simulated 2DES maps at the three different excitation conditions are reported in Fig. 3d–f. Clearly, the simulated maps could not reproduce the strong ESA signal recorded in the experiments, as the S_1 state is not included in the simplified model. Nevertheless, the simulations are able to capture the Q_y main features and the intertwined dynamics between Q_y and S_2, supporting the main components within the distribution of EET rates, which are characterized by an average time constant of approximately 63 fs, seen to be notably affected by coherent mechanisms. These include a nontrivial sink effect by vibrational modes of the Per, which may contribute to the high efficiency of this relaxation pathway. In fact, we recorded the evolution of the coherent superposition of S_2 and Q_y detected as a beating frequency with ~20 fs dephasing time. The evolution of this coherence, prepared by the laser pulse, appears functional to the EET, since a steep rising of the Q_y diagonal signal, justifiable only with coherent pathways, has been recorded within the same timescale. This suggests that the evolution of the coherence effectively moves the population on the Q_y state. Our data and calculations suggest that...

Discussion

We have reported here 2DES measures recorded in different experimental conditions, analyzed with state-of-the-art data analysis methods, and supported by multiscale quantum chemical calculations on the pigment–protein complex. These measures allowed a thorough and complete mapping of the multiple pathways mechanism of energy flow in PCP, and the relative time constants. The picture emerging from computational and experimental data is that contrary to that generally assumed, the energy flow from Per to Chl in the investigated time and spectral windows, is dominated by a direct channel involving Q_y state of Chl as acceptor and the S_2 state of the Per as donor. These findings represent a further insight toward the full resolution of a long-term discussion about the effective states involved in the energy flow from Per to Chl. More importantly, the fast components within the distribution of EET rates, which are characterized by an average time constant of approximately 63 fs, seem to be notably affected by coherent mechanisms. These include a nontrivial sink effect by vibrational modes of the Per, which may contribute to the high efficiency of this relaxation pathway. In fact, we recorded the evolution of the coherent superposition of S_2 and Q_y detected as a beating frequency with ~20 fs dephasing time. The evolution of this coherence, prepared by the laser pulse, appears functional to the EET, since a steep rising of the Q_y diagonal signal, justifiable only with coherent pathways, has been recorded within the same timescale. This suggests that the evolution of the coherence effectively moves the population on the Q_y state. Our data and calculations suggest that...
in the most coupled Per-Chl pair, partially delocalized states are created upon photoexcitation and they dephase quickly, localizing population on the final acceptor state. Thus, the presence of coherence appears functional to the energy distribution in PCP. This is important information in current efforts to evaluate possible opportunities to harness coherence to realize, control, and/or drive energy transduction. While our results refer here to a specific protein, nevertheless they demonstrate that the transfer from Cars to Chl can sustain coherent dynamics also when the spectral detuning seems to be not fully favorable. This is an important piece of information in the current efforts of unveiling the still unclear mechanism of energy flow from Cars to Chl.

Methods

Two-dimensional electronic spectroscopy. We performed 2DES experiments in the fully non-collinear BOXCARS geometry, employing the setup described in ref.47. The 2DES experiments were repeated in three different experimental conditions: the laser spectrum was centered at 15 600 (laser 1), 15 900 (laser 2), and 16 200 cm\(^{-1}\) (laser 3), respectively, with \(-2000\) cm\(^{-1}\) full-width at half maximum. The pulse duration was determined by FROG measurements at the sample position: the broadband pulses were compressed to 11.0, 8.9, and 8.5 fs with laser 1, 2, and 3, respectively (Supplementary Fig. 15). They were attenuated to 5 nJ pulse by a broadband half-waveplate/polarizer system. All measurements were performed under room temperature (295 K). Population time was scanned up to 1 ps with 5 fs time step and coherence time from --80 to 100 fs in 1 fs steps. Each experiment was repeated and averaged three times to ensure reproducibility.

Data analysis was performed exploiting the global fitting methodology described in ref.35 using a kinetic model to detail the populations dynamics. The first 10 fs have been omitted in the analysis to avoid pulse overlap artifacts.

Sample preparation. PCP antenna complex was extracted and purified by Professor R. G. Hiller from Amphidinium carterae, according to the method previously published in ref.48. For the 2DES experiments, the sample was diluted in sodium phosphate buffer (pH 7.5) until an optical density of about 0.4 in a 1 mm cuvette was reached in the region of the Q band.

The sample was accurately degassed under nitrogen flux to remove the oxygen from the solution and the cell was sealed immediately after to avoid the formation of oxidized species during 2DES measurements.

Quantum chemical calculations. The crystal structure of PCP was refined by relaxing the geometries of the pigments through a quantum mechanics/molecular mechanics (QM/MM) optimization in a frozen protein environment. The excited states of the complex are described through an exciton model that comprises the S\(_2\) bright states of the eight Per, plus the Q and B states of Chls, resulting in 16 total states. The electronic couplings among all excited states were computed with an approach based on the transition densities of interacting pigments. All excited-state calculations were performed using a multiscale polarizable QM/MM scheme (QM/MMPol). Within this framework, the protein (and the other cofactors) surrounding the pigment of interest are described through a set of atomic point charges and isotropic polarizabilities. The QM/MMPol scheme accounts for mutual polarization effects between the pigments and the environment; moreover, it allows the environment to respond to the excitation of the pigment and to properly describe the dielectric screening due to the anisotropic environment of the protein in the calculation of the electronic couplings. For all calculations, we used the (TD)-DFT\(^*\) method with the B3LYP functional. Spectra and dynamics were modeled with the disordered exciton model, using the second-order cumulant expansion with modified Redfield theory. Further details are presented in the Supplementary Methods 1.

Theoretical 2DES spectra. The 2DES signal of PCP was simulated using the excitonic heterodimer model reported in ref.53. Excited-state Q\(_S\) of Chl602 and S\(_2\) of Per624 were considered, exerting an electronic coupling of 308.4 cm\(^{-1}\) computed in the full excitonic model. The simulation of the third-order optical response was fully fed with parameters computed in the quantum chemical calculations. The strong inhomogeneous broadening of Per system asked for an averaging of the optical properties over 200 disorder realizations. Each snapshot was characterized by specific site energies, transition dipole moments, lineshape functions, and transfer rates (Supplementary Notes 1 and 2). Rephasing and non-rephasing impulsive contributions were fully convolved with the experimental laser spectrum, in order to match the experimental signals.

Data availability. All data supporting the findings of this study are available from the corresponding author upon request. All custom codes used in this study are available from the corresponding author under reasonable request.

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Author contributions
E.M., A.V. and L.B. performed spectroscopic measurements and data analysis; L.C., S.I. 
and V.M. performed QM calculations; E.C. and B.M. designed the research; E.C., B.M., 
and D.C. helped with data interpretation. E.M., A.V., E.C., L.C. and B.M. wrote and 
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