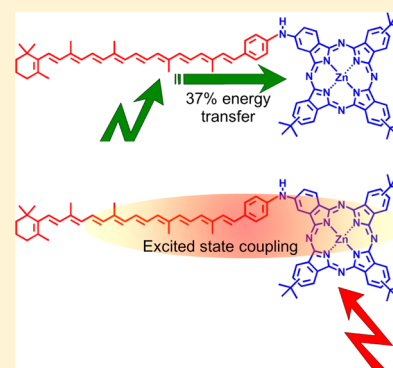


Ultrafast Energy Transfer and Excited State Coupling in an Artificial Photosynthetic Antenna

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S Supporting Information

ABSTRACT: We have studied the energy transfer dynamics in an artificial light-harvesting dyad composed of a phthalocyanine (Pc) covalently linked to a carotenoid (Car). The combination of high temporal resolution transient absorption spectroscopy with global and target analysis allowed us to quantify the efficiency of the energy transfer from the S_2 excited state of the Car to the Pc at 37%, close to values observed in some natural light-harvesting complexes. In addition, following selective excitation of the Pc, we have identified the spectral signatures of the S_1 excited state of the Car which appear within the ≈ 30 fs time resolution of our measurement. This strongly indicates excited state coupling between the S_1 state of Car and the Q_x state of Pc, with important implications for the regulation of photosynthetic activity.



■ INTRODUCTION

Photosynthetic light harvesting involves the absorption of sunlight to create electronic excitations in the peripheral antennas of photosynthetic systems and the subsequent rapid excitation energy transfer (EET) to a reaction center.^{1–3} EET occurs through highly engineered, sophisticated, and extensive networks of pigment–protein complexes, made of densely packed chromophores held in precise configurations by the surrounding protein scaffold. The chromophores present in natural antennas are carotenoids (Cars) and (bacterio)-chlorophylls ((B)Chls). Cars absorb light in the blue-green region of the spectrum and transfer the electronic excitation to the (B)Chls, thereby increasing the overall efficiency of the light-harvesting process. In addition, Cars play crucial roles in photoprotection of the photosynthetic apparatus.⁴ First, they scavenge the highly reactive singlet oxygen and limit its generation by quenching its precursor (B)Chl triplet state. Second, they are involved in the so-called nonphotochemical quenching (NPQ) mechanism,^{5,6} which allows the photosynthetic organisms to adapt to the changing light levels by downregulating their photosynthetic activity under high illumination conditions. Although the precise mechanism of NPQ is still under debate and may vary from one system to another,^{7–10} there is now a general agreement that it occurs by quenching the Chl singlet states via interaction with Cars.

The main features of Car photophysics can be described by a model based on two low-lying excited singlet states, denoted S_2 and S_1 .^{11,12} In this picture, the transition from the ground state

S_0 (with $1^1A_g^-$ symmetry) to the excited state S_2 ($1^1B_u^+$) is optically allowed and leads to a strong absorption band in the visible region. The transition from S_0 to the lower-lying S_1 ($2^1A_g^-$) state is one-photon-forbidden for reasons of symmetry. Following photoexcitation, energy relaxes from the S_2 state into the S_1 state by internal conversion (IC) in a few hundreds of femtoseconds; then, S_1 decays back to S_0 on a slower, picosecond time scale.¹³ Within the photosynthetic apparatus, EET from Cars to (B)Chls can occur from both S_2 and S_1 states.¹⁴ Despite extensive investigations, the details of the electronic structure of carotenoids are still a matter of ongoing debate.¹⁵ Recently, a growing number of experiments on Cars, both in solution and within light harvesting complexes call for a scenario which challenges the simple three-level picture, with additional excited states^{16–18} required for a full description of their photoinduced dynamics.

Synthetic chemistry has made possible the development of artificial complexes which mimic many of the functions of natural light harvesting.^{19,20} In these systems the chromophores, i.e., Cars and tetrapyrroles (porphyrins/phthalocyanines), are linked by covalent bonds rather than being held in position by the protein scaffold. The study of such artificial antennas is interesting on the one hand because they allow the investigation of the fundamental mechanisms of natural light-

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harvesting while working on simpler and more controlled systems. On the other hand, they can be considered as the main building blocks of future bioinspired artificial systems that can be used to convert light into stored chemical energy.²¹

In this work we focus on a prototypical artificial dyad, which mimics the light harvesting process in natural photosynthesis, in order to address two fundamental roles of Cars in photosynthesis: EET and photoprotection via NPQ. We study a dyad composed of a Car with 10 conjugated double bonds, serving as light harvester, linked to a phthalocyanine (Pc), acting as energy acceptor, through a phenylamino group, resulting in an effective conjugation length $N \approx 11$. Recently, the same artificial complex has been studied by Kloz et al.^{22,23} in different solvents, but information about the initial events that occur directly after photoexcitation was limited by the 100 fs time resolution of the conventional transient spectrometers employed.

Our work extends previous studies on this dyad in two important ways: (i) we significantly improve the temporal resolution, using an advanced pump–probe setup with sub-20 fs pulses; (ii) we combine the high quality experimental data with a modeling approach that uses global and target analysis, with the additional requirement that different data sets on the dyad and its isolated moieties are fitted simultaneously.

Thanks to the ≈ 30 fs temporal resolution of our measurements, we are able to resolve the dynamics of the extremely fast Car \rightarrow Pc EET and $S_2 \rightarrow S_1$ IC processes, not observed in ref 23 due to the limited 100 fs time resolution. In addition, upon selectively pumping the Pc with sub-20 fs pulses, we detect the instantaneous appearance of transient features characteristic of the Car S_1 state, confirming the results of ref 22 but with 3 times better time resolution. The observation of an instantaneous Car signal upon photoexcitation of the Pc strongly suggests the existence of excited state coupling between the Q_x state of the Pc and the S_1 state of the Car. Taken together, our results highlight the similarity between artificial and natural supramolecules in terms of both light-harvesting and photoprotective functions.

MATERIALS AND METHODS

Synthesis. The chemical structure of the dyad is shown in the inset of Figure 1. The dyad and the two isolated reference compounds, Pc and Car, were synthesized as previously described.²⁴ The dyad consists of a 10-double-bond polyene terminated with a *para*-phenylene group that is attached to an amino group at a peripheral position of Pc. The reference Pc is identical to the dyad, but the polyene chain is replaced by a methyl group and in the reference carotenoid the Pc moiety of the Car-Pc is replaced by a tolyl group. The dyad actually exists as a mixture of isomers in which the positions of the various *tert*-butyl groups vary. This is not expected to significantly affect the photophysics of the molecule.

The dyad and its two reference compounds were dissolved in toluene for the spectroscopic measurements described below. The ground-state absorption spectrum of the dyad is reported in Figure 1 as a solid black line: the Car $S_0 \rightarrow S_2$ transition lies in the blue-green (450–550 nm wavelength region), while the Pc moiety has its main absorption peak at ≈ 690 nm (due to its Q_x transition), with a small replica at ≈ 625 nm (due to its Q_y transition). We note that in PCs the Q_x/Q_y name ordering is reversed as compared to (B)Chls.

Ultrafast Spectroscopy. The high time resolution experimental apparatus is based on two synchronized noncol-

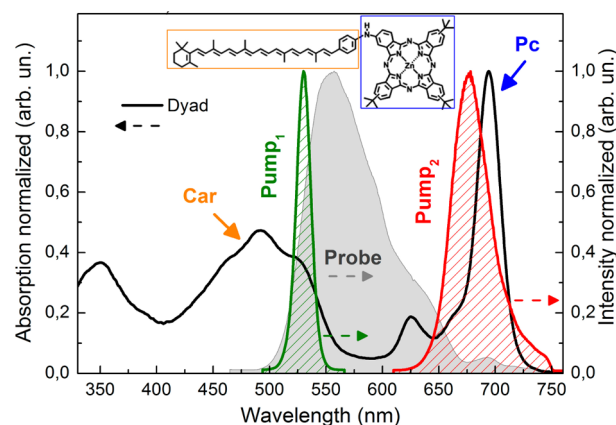


Figure 1. Chemical structure and absorption spectrum (black solid line) of Pc-Car dyad diluted in toluene and probe spectrum (gray area) used in the experiments; Pump₁ and Pump₂ represent the different pump pulses used for excitation at 530 and 680 nm (green and red dashed curves, respectively).

linear optical parametric amplifiers (NOPAs)²⁵ pumped by a regeneratively amplified mode-locked Ti:sapphire laser system delivering pulses with 150 fs duration, 500 μ J energy, at 1 kHz repetition rate and 780 nm central wavelength. The first NOPA generates 15 fs pulses peaked at 530 nm (Pump₁ in Figure 1), in resonance with the $S_0 \rightarrow S_2$ transition of the Car. The same NOPA can be tuned to generate 15 fs pulses at 680 nm (Pump₂ in Figure 1) in order to selectively excite the Pc. The second NOPA provides ultra-broadband probe pulses with ≈ 7 fs duration spanning the 500–700 nm wavelength range (gray filled spectrum in Figure 1), thus covering both the Car and Pc absorption bands. Both NOPAs are compressed to their transform-limited duration by multiple bounces on custom-designed chirped mirrors. The beams are focused onto the sample contained in a cuvette with 200 μ m thick fused-silica windows. After the sample, the probe beam is selected by an iris and focused onto the entrance slit of a spectrometer with single-shot detection capability at 1 kHz.²⁶ By recording the probe spectrum with and without the pump, one can obtain the transient absorption (TA) spectrum, defined as $\Delta A(\lambda, \tau) = -\ln[T_{\text{on}}(\lambda, \tau)/T_{\text{off}}(\lambda)]$, as a function of probe wavelength and delay.

Data Analysis. For this study a total of four measurements (Car and dyad with excitation at 530 nm, Pc and dyad with excitation at 680 nm) were analyzed by a combination of global and target analysis.²⁷ Software to perform this kind of analysis is freely available in the form of the Glotaran software package.²⁸ The TA spectra of the dyad and the isolated Car excited at 530 nm were recorded in a 50 ps time window and then analyzed simultaneously using a specific kinetic scheme (target analysis), resulting in the estimated species associated difference spectra (SADS) of the pure molecular states. For this set of measurements the instrument response function (IRF) was fitted to a Gaussian of 42 fs full width at half-maximum (fwhm) for both samples.

With 680 nm excitation the TA spectra of the dyad and the isolated Pc were recorded in a 100 ps time window and then analyzed simultaneously using a sequential kinetic model ($1 \rightarrow 2 \rightarrow 3 \rightarrow \dots$) in which the arrows indicate successive monoexponential decays with increasing time constants (global analysis). Each component is characterized by its evolution associated difference spectrum (EADS). The first EADS then

corresponds to the time-zero TA spectrum. Only three components were required to describe the spectral evolution in the 100 ps measurement window. Because no detailed kinetic scheme is imposed, the EADS may reflect mixtures of molecular species, and in fact for the dyad this is most certainly the case as is explained in the Results section. For this set of measurements the IRF was fitted to a Gaussian of ≈ 60 fs fwhm for both the Dyad and the Pc samples. Given the IRF and the signal-to-noise ratio of our data, we can conservatively estimate a time resolution of at least 30 fs.

RESULTS

Carotenoid–Phthalocyanine Energy Transfer. The Car \rightarrow Pc EET process in the dyad can in principle occur from the bright S_2 excited state as well as from the dark S_1 state of the Car; in both cases, it competes with IC processes toward lower-lying states. To establish the relative weight of these two deactivation pathways, we compare the excited state dynamics of the isolated Car (where only the IC process occurs) with that of the dyad (where both the IC process and the EET channel are active).

Figure 2a shows the ΔA heat map of the isolated Car in toluene solution: we observe the prompt rise of a negative

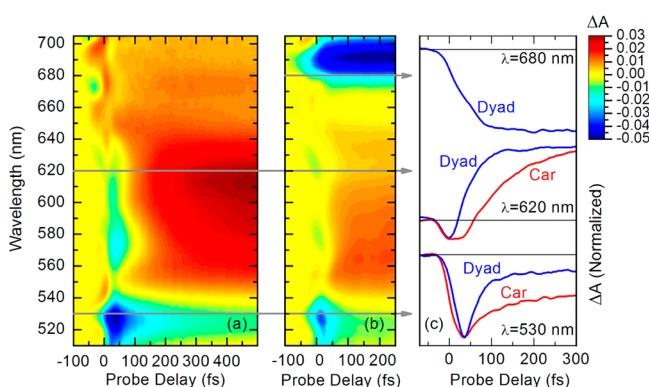


Figure 2. ΔA heat maps as a function of probe wavelength (vertical) and probe delay (horizontal) for the isolated Car (a) and the dyad (b) in toluene; excitation at 530 nm. As indicated in the color map, the zero level is colored in yellow, red indicates positive signals (i.e., photoinduced absorption), and green/blue denote negative signals (i.e., decrease in absorption due to stimulated emission and/or ground-state photobleaching). Time traces at selected probe wavelengths (c).

signal, which we assign to ground state photobleaching (PB) and to stimulated emission (SE) from S_2 to S_0 . This signal rapidly decays, giving rise to a positive photoinduced absorption (PA) band peaking at 610 nm, the formation of which is completed within ≈ 400 fs. This PA band is well-known in Cars and assigned to a transition from S_1 to a higher-lying S_n state,¹³ thus providing a signature of the population of the S_1 state through the IC process. The PA band shows a faster buildup in the red compared to the blue and undergoes spectral narrowing within the first 500 fs. These effects are due to intramolecular vibrational relaxation (IVR) within S_1 associated with dissipation of the excess energy deposited as a result of the IC process.^{29,30}

Figure 2b shows the ΔA heat map of the dyad in toluene solution, following excitation of the Car moiety at 530 nm. In this case, the temporal evolution of the TA signals occurs on a significantly shorter time scale as compared to the isolated Car,

indicating a much faster decay of the population out of the initially excited Car S_2 state (see comparison of time traces in Figure 2c). In particular, within ≈ 100 fs following excitation a nearly stationary TA spectrum is formed, which displays not only the expected $S_1 \rightarrow S_n$ PA from the Car but also (i) a negative PB signal peaking at around 690 nm, reflecting excitation of the Pc moiety and thus indicating Car \rightarrow Pc EET, and (ii) an increased PA shoulder in the blue (peaking at ≈ 555 nm), representing a new deactivation pathway within the Car manifold toward an additional intermediate excited state, denoted as S^* . A spectrally similar state, which in our experiments is not observed in the isolated Car (Figure 2a), has been observed in other Cars in the same spectral region, both in solution¹⁵ or when embedded in light-harvesting systems.^{31–33}

Figure 3a depicts the compartmental model used for the target analysis of the 530 nm excitation data, using the minimum number of components that were necessary to satisfactorily fit the data. Alternative models were initially considered and investigated and then discarded based on the decreased quality of fits and/or physical interpretability of the estimated parameters (rate constants and SADS). S_2 , hot S_1 , S_1 , and S^* are SADS associated with Car, while Pc1 and Pc2 are associated with the phthalocyanine. Spectral evolutions of hot $S_1 \rightarrow S_1$ and Pc1 \rightarrow Pc2 were taken into account in order to include IVR processes within the two species: as a consequence of $S_2 \rightarrow S_1$ IC or Car \rightarrow Pc EET, excess energy is initially deposited both in the Car S_1 state and in the Pc, due to the excitation of the Car S_2 state via the 530 nm pump pulse. Both for the isolated Car and for the dyad the model did not require the inclusion of additional excited states of the Car (such as the $1^1B_u^-$ state), which in some cases have been proposed to mediate the $S_2 \rightarrow S_1$ IC process.^{16–18} The estimated population time profiles and corresponding SADS are shown in Figures 3b and 3c, respectively. Figure S1 reports the same SADS of Figure 3c but vertically offset to improve readability. Car $S_2 \rightarrow$ hot $S_1 \rightarrow S_1 \rightarrow S_0$ IC rate constants and associated SADS were constrained to be equal in the analysis of the isolated Car and the dyad. This approach is based on the assumption that when covalently linking the two molecules, their excited states are not significantly affected. Without this assumption it would be very difficult to compare the experiments in the dyad with those of the isolated moieties. The first SADS can be identified as the S_2 state of the Car, with a PB at 525 nm and SE from 550 until 650 nm. From the S_2 state of the Car three different species are populated in the dyad: the hot S_1 of the Car, the first excited state of the Pc species (Pc1), and the additional excited state S^* of the Car. The lifetime of the S_2 state is 39 ± 2 fs in the dyad. The S^* state subsequently decays in 320 ± 10 fs to the ground state, presumably via an IC process. Based on previous results,^{31–34} the Car S^* state is restricted to only contribute to the fit for wavelengths shorter than 585 nm. The hot S_1 state evolves in ≈ 440 fs into the relaxed S_1 state, which subsequently decays to the ground state in 7.5 ps. Analogously, the hot Pc (Pc1) evolves in ≈ 3 ps into the relaxed Pc state (Pc2), which relaxes to the ground state on a much longer time scale. The excellent fit quality of the simultaneous target analysis is demonstrated in Figure S2.

Carotenoid–Phthalocyanine Coupling. To investigate the possible coupling between the excited states of the Pc and Car moieties in the dyad, we compare TA measurements on the dyad with those of the isolated Pc, both excited at a wavelength of 680 nm, corresponding to the peak of Pc absorption and

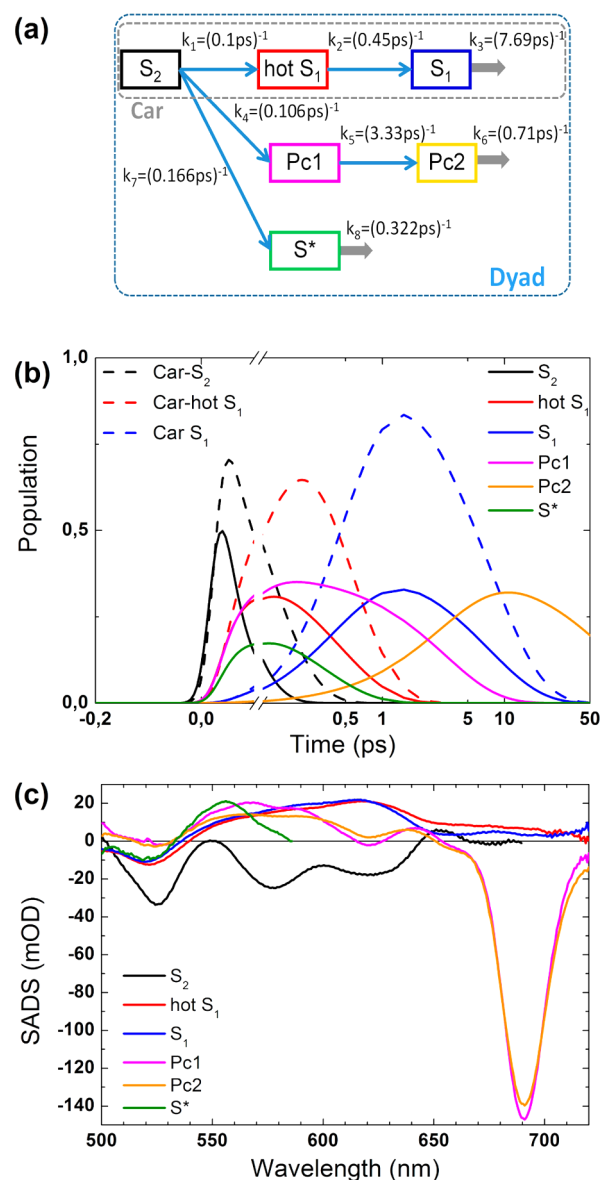


Figure 3. (a) Kinetic scheme used for target analysis of the 530 nm excitation data. The estimated rate constants (in $1/\text{ps}$) are indicated in the figure; the global lifetimes are 0.1 ps (S_2 , Car), 0.039 ps (S_2 , dyad), 0.44 ps (hot S_1), 7.5 ps (S_1), 3.3 ps ($Pc1$), 73 ps ($Pc2$), and 0.32 ps (S^*). (b) The population profiles and (c) estimated SADS. Note that the time axis is linear until 0.1 ps and logarithmic thereafter. The dashed population profiles in (b) correspond to the isolated Car and the solid ones to the Car–Pc dyad.

with negligible absorption from the Car. Figure 4 reports the EADS obtained from the simultaneous global analysis of both measurements; the raw data in the form of kinetic traces and fits are reported in Figure S3.

The interpretation of these EADS is quite straightforward. Upon 680 nm excitation of the isolated Pc molecule, the photogenerated excited state $Pc1$ with a 94 fs time constant undergoes a tiny red-shift, attributed to IVR, evolving to $Pc2$, which decays in 54 ps into a long-lived component $Pc3$ which does not evolve in our time window. IVR occurs because the pump spectrum is broader than the Pc absorption and the excitation pulse peaks at a wavelength slightly blue-shifted with respect to the Pc absorption maximum (see Figure 1). The dyad undergoes a similar evolution on the same time scale, but

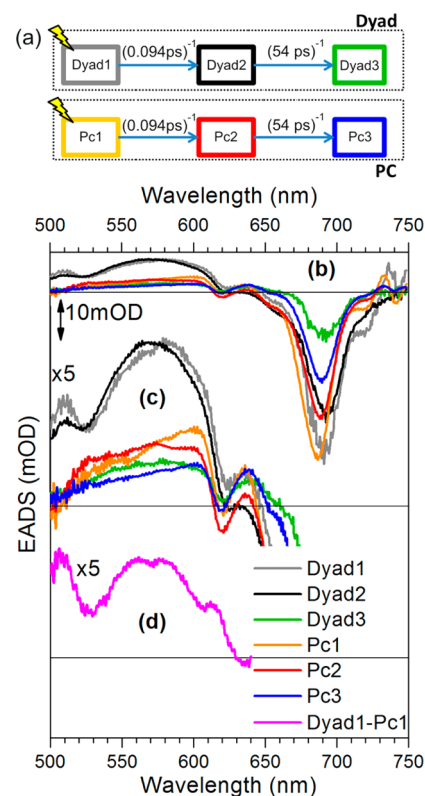


Figure 4. (a) Kinetic scheme used for target analysis of the 680 nm excitation data. The dynamics was modeled with three sequential decays. The time constants were linked between the experiments and found to be 0.094 ps (for $Pc1$ and $Dyad1$), 54 ps (for $Pc2$ and $Dyad2$), and long-lived (for $Pc3$ and $Dyad3$). (b) EADS resulting from the simultaneous analysis of the measurements collected for the isolated Pc moiety ($Pc1$, $Pc2$, and $Pc3$; yellow, red, and blue curves) and the dyad ($Dyad1$, $Dyad2$, and $Dyad3$; gray, black, and green curves) at 680 nm excitation. (c) EADS magnified 5 times. (d) $Dyad1$ EADS minus the $Pc1$ EADS (magenta curve).

with the PB signals of the Pc moiety in the dyad clearly red-shifted by approximately 5 nm with respect to the isolated Pc molecule. The same red-shift is observed in the ground-state absorption spectrum and is assigned to the effect of the linkage joining the Pc to the carotenoid. For the isolated Pc, the EADS show two PB bands, at ≈ 690 and ≈ 620 nm, assigned to the Q_x and Q_y transitions, respectively, and a broad and almost featureless PA band extending to the blue.

The EADS for the dyad are qualitatively similar but, as shown expanded in the middle panel, with clear differences in the PA signal. To better highlight these differences, we subtracted the isolated Pc EADS from the dyad EADS (panel c). When this difference EADS (pink curve in Figure 4d) is compared qualitatively with the estimated SADS from Figure 3c, it is clear that the extra spectral signature observed in the dyad (i.e., a PA signal between 540 and 620 nm) closely matches the Car S_1 excited state TA spectrum (blue SADS in Figure 3c). We also observe additional negative features around 530 nm and around 630 nm. The former corresponds to the PB of the Car $S_0 \rightarrow S_2$ transition, while the latter can be explained by a shift of the Q_y absorption band of the Pc in the dyad. All these additional features in the dyad EADS do not display any rise time within our ≈ 30 fs temporal resolution but are immediately present following photoexcitation of the Pc moiety. As discussed below,

we take them as a clear evidence of electronic coupling between the Pc excited state and the S_1 excited state of the Car.

DISCUSSION

Carotenoid–Phthalocyanine Energy Transfer. EET between Cars and (B)Chls in natural light-harvesting complexes has been studied in detail using ultrafast spectroscopy, as recently reviewed by Polivka and Frank.¹⁴ At first sight, one would expect EET from Cars to be a rather inefficient process. On the one hand, the S_2 lifetimes are rather short (ranging from 50 to 300 fs depending on the number of double bonds N), providing an efficient parallel deactivation channel via IC to S_1 , and on the other hand, the $S_0 \rightarrow S_1$ transition is optically forbidden, so that one would expect that direct dipole-coupled Förster transfer from S_1 should be inefficient. However, the S_2 EET channel is always found to be active, while the S_1 channel is activated only in specific systems. The reason for the high efficiency of EET from S_2 lies in the optimization of the Car–(B)Chl distance, which, combined with the large value and proper orientation of the S_2 dipole moment, results in rather large Förster EET rates.³⁵ The most studied systems for EET are the peripheral LH2 complexes of purple bacteria due to the availability of detailed structural information and to the well-separated absorption spectra of the different pigment groups. In the prototypical purple bacterium *Rhodospseudomonas (Rps.) acidophila*, containing the carotenoid rhodopin glucoside ($N = 11$), EET has been investigated in many studies, and its efficiency has been found to be around 50% from S_2 .^{30,36,37} In other bacteria this efficiency has been found to be different. When the Car conjugation length increases, the S_2 lifetime becomes shorter, thus reducing the EET efficiency. For our dyad, the EET efficiency from the Car S_2 state to the Pc can be calculated as $\eta_{ET2} = k_4/(k_1 + k_4 + k_7) = 37\%$, highlighting the similarity of our artificial antenna with natural light-harvesting complexes.³⁸ This number agrees very well with independently performed fluorescence excitation measurements where the energy transfer efficiency for this system was found to be 42% integrated over the relevant spectrum (Gerdenis Kodis (ASU), private communication). Without the loss channel from S_2 to S^* (k_7 in Figure 3a) the energy transfer efficiency would be higher than 60%.

In some purple bacteria the EET channel from S_1 is activated because the Car to BChls distance is smaller than the molecules themselves, so that the charge density locally changes and the Förster transfer becomes permitted (one should consider the full donor/acceptor Coulomb coupling). In particular, it is found to be quite efficient for bacteria having Cars with $N < 11$, while it is not significant for longer Cars.^{14,39} We considered EET from S_1 to Pc in our dyad molecule, but we found this contribution not to be present: the S_1 dynamics were found to be unchanged between the isolated Car and the dyad. This means that no EET process occurs from the S_1 state of the Car, i.e., $\eta_{ET1} \cong 0$. We note that with respect to LH2 complexes, where the first excited state Q_y of the accepting BChl molecule absorbs at 800/850 nm, here the Pc lower state (Q_x) is higher in energy (it absorbs at ≈ 690 nm). The Car S_1 state energy (measured in many samples using fluorescence, two-photon absorption, Raman or infrared S_1 – S_2 absorption) depends on the conjugation length (the higher N , the lower its energy). For example, in spheroidene having $N = 10$, the S_0 – S_1 gap would correspond to a one-photon transition at 700–750 nm.¹³ In the dyad, the phenyl group linkage between the Car and Pc results in an increased conjugation length, thus further decreasing the

S_0 – S_1 gap. This points to an unfavorable uphill energy landscape for the $S_1 \rightarrow$ Pc EET.

The TA data in the dyad clearly show an additional decay pathway for the S_2 state of the Car, toward an excited state resembling S^* . The S^* state was discovered about a decade ago in spirilloxanthin ($N = 13$),³¹ both in solution and bound to the LH1 complex of *Rhodospirillum rubrum*, and later observed in other carotenoids.^{31,32,40,41} It is characterized by a PA band blue-shifted with respect to the S_1 – S_n band but red-shifted with respect to the optically allowed S_0 – S_2 transition. Despite alternative assignments of S^* to a hot ground state,^{33,42} there is now a general consensus in interpreting it as a separate excited state, generated from S_2 in competition with S_1 , and often leading to the formation of a long-lived triplet state in Cars.^{43,44} Generally, S^* is found to have a longer lifetime with respect to S_1 ; here, on the other hand, its lifetime (0.3 ps) is measured to be significantly shorter than the S_1 lifetime (7.5 ps). Niedzwiedzki et al.⁴⁵ considered the hypothesis of S^* as a distorted S_1 state, but more research is needed to decide on the nature of this short-lived S^* state. We note that such short-lived S^* state, acting as a quencher for the carotenoid S_2 state, could play an important role in the carotenoid/(bacterio)chlorophyll interaction found in photosynthetic bacteria and higher plants. The fact that we do not detect it in the isolated carotenoid could indicate that fine energetic tuning could be actively used in nature (maybe even in the NPQ mechanism) to activate or suppress this energy deactivation channel. Alternative target models, considering the absence of S^* state in the dyad or its presence also in the Car, were considered (see Figures S4 and S5 in the Supporting Information and relative discussion) but discarded based on the decreased quality of fit and the physical interpretability of the estimated parameters.

Carotenoid–Phthalocyanine Excited State Coupling.

NPQ is the mechanism employed by plants and algae to self-protect from the adverse effects of high light intensity. It is based on the quenching of Chl singlet excited states via enhanced nonradiative internal conversion to the ground state. The molecular processes underlying NPQ are not yet fully understood and are hotly debated, but there is a general agreement regarding the fact that Car–Chl electronic interactions are involved. A possible pathway is EET from Chl to the S_1 dark state of the lutein carotenoid, enabled by conformational changes in the peripheral, trimeric major light-harvesting antenna of photosystem II, called LHCII.⁷ Alternatively, a charge transfer mechanism has been proposed, with charge separation followed by recombination occurring in the minor antenna complexes after energy transfer to a Chl/zeaxanthin heterodimer.^{8,9} Recently in LHCII the formation of coupled excitonic states between the S_1 state of the Car and the Chl, which decrease the excited state lifetime of Chl, has been proposed to mediate NPQ.⁴⁶

In order to shed light on the mechanisms of NPQ in natural systems, it is interesting to investigate an artificial dyad such as the one addressed in this study as a model system containing only one Car and one tetrapyrrole unit. The advantage resides in the fact that in this case the pure quenching kinetics can be directly studied because they are shorter than the decay times of the tetrapyrrole excited states by their intrinsic photophysical pathways. On the other hand, in the natural light-harvesting systems, such kinetics are obscured because the excitation must first slowly transfer (in hundreds of picoseconds) from the large pigment pool to the Cars/Chls in the quenching site, where it rapidly decays. This results in an inverted kinetics, for which the

TA signal from the Car S_1 state is small and convoluted with the exciton dynamics in the pigment pool.

In Car/tetrapyrrole dyads all the three aforementioned quenching mechanisms have been observed, depending on the exact molecular architecture and solvent environment:

(1) EET from Pc to the S_1 state of the Car, possibly passing through an intramolecular charge-transfer state, followed by fast internal conversion to the Car ground state;^{47,22} this occurs only for carotenoids with sufficiently large conjugation length to lower the S_1 energy, analogously to the “molecular gear shift mechanism” proposed for the xanthophyll cycle.⁴⁸

(2) Electron transfer from Car to Pc followed by rapid charge recombination on the picosecond time scale, occurring especially in polar solvents.^{20,49}

(3) Excitonic coupling between the Pc and the S_1 state of the Car and ensuing internal conversion to the ground state of the Car. This mechanism has been proposed to occur in the Pc/Car dyad that we are studying on the basis of two complementary experimental results: (i) in a pump–probe study, upon Pc excitation the TA signal from the Car S_1 state immediately appeared within the 100 fs instrumental temporal resolution, indicating $Pc \rightarrow Car$ interaction;²² (ii) in a fluorescence experiment, upon direct Car S_1 population (using two-photon excitation) a strong emission from Pc was observed, indicating $Car \rightarrow Pc$ interaction.⁵⁰ Such a bidirectional $Car \leftrightarrow Pc$ EET evidence excitation energy sharing between the two moieties and is a very strong indication for excited state interactions. Such excited state coupling ultimately depends on a delicate balance of the Car S_1 /Pc relative energies and on thermal fluctuations. In more polar environments (THF) such coupled states have been observed and tentatively assigned as precursors of charged-separated states.²²

The experimental results presented in Figure 4c clearly show that upon Pc excitation an additional TA signal immediately appears in the dyad with respect to the pure Pc sample. With the help of our target analysis reported in Figure 3c we can clearly identify this band as PA from the Car S_1 state (blue SADS in Figure 3c). We also observe PB of the Car $S_0 \rightarrow S_2$ transition at 530 nm. These bands appear within our temporal resolution of ≈ 30 fs, corresponding to a 3-fold improvement with respect to ref 22. Three possible scenarios could explain this observation: (i) extremely rapid $Pc \rightarrow Car$ S_1 EET, which would result in equally rapid deactivation of Pc, not observed in our experiments; (ii) $Pc \rightarrow Car$ S_1 EET slower than Car S_1 deactivation, resulting in an inverted kinetics; in this case, however, the S_1 signal would rise with a time constant corresponding to the S_1 lifetime, which is much longer (on the several picoseconds time scale, see Figure 3a,b) than our temporal resolution; (iii) excited state coupling between Pc and Car S_1 , which is then our proposed explanation.

CONCLUSION

In this work we performed a comprehensive study of excited state dynamics in a carotenoid–phthalocyanine dyad which acts as an artificial photosynthetic antenna. By the use of our state-of-the-art pump–probe system with high temporal resolution, it was possible to completely time resolve the ultrafast excited-state dynamics of the isolated Car and of the dyad system, occurring within the ≈ 100 fs time scale. The combination of experimental results and their simultaneous comparison by means of global and target analysis enabled us to evaluate the EET pathways and efficiency (as high as $\approx 37\%$) from the Car to the Pc. In addition, by exciting Pc moiety in the dyad, we

observe, within the ≈ 30 fs time resolution of the experiments, features in the transient absorption spectrum which closely match those of the S_1 excited state of the Car. This result strongly suggests that in this dyad there is electronic coupling between the Pc excited state and the S_1 excited state of the Car, a mechanism that has been recently proposed to mediate NPQ in the LHCII complexes of higher plants. Excited state coupling has been reported in several natural photosynthetic systems such as Fenna–Matthews–Olson (FMO) bacteriochlorophyll complex,⁵¹ light-harvesting proteins isolated from marine cryptophyte algae,⁵² and the trimeric light-harvesting complex II (LHCII)⁵³ from *Arabidopsis thaliana* using two-dimensional electronic spectroscopy (2DES). Oscillations were observed in these experiments and interpreted as a long-lived superposition (coherence) of exciton states. These observations were made possible by the fact that 2DES signals are recorded at the amplitude level, so that the quantum phase of the system can be directly accessed, thereby providing evidence of quantum coherence effects. For these reasons, the dyad system studied in this work is an ideal candidate for future 2DES measurements: the excitonic coupling between the carotenoid and the PC we observed here using pump–probe spectroscopy could in principle manifest itself as a coherence in the 2DES signal.

This study highlights once again the similarity between artificial and natural photosynthetic complexes in terms of two fundamental functions: light harvesting and photoprotection. These results show that artificial complexes are promising for future application for controlling natural photosynthesis.

ASSOCIATED CONTENT

Supporting Information

Supplementary figures (including a complete comparison of data and fits) and supplementary discussions (analysis of alternative target models). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) van Amerongen, H.; Valkunas, L.; van Grondelle, R. *Photosynthetic Excitons*; World Scientific: Singapore, 2000.

- (2) van Grondelle, R.; Novoderezhkin, V. I. Energy Transfer in Photosynthesis: Experimental Insights and Quantitative Models. *Phys. Chem. Chem. Phys.* **2006**, *8*, 793–807.
- (3) Novoderezhkin, V. I.; van Grondelle, R. Physical Origins and Models of Energy Transfer in Photosynthetic Light-Harvesting. *Phys. Chem. Chem. Phys.* **2010**, *12*, 7352–7365.
- (4) Frank, H. A.; Cogdell, R. J. Carotenoids in Photosynthesis. *Photochem. Photobiol.* **1996**, *63*, 257–264.
- (5) Niyogi, K. K. Safety Valves for Photosynthesis. *Curr. Opin. Plant Biol.* **2000**, *3*, 455–460.
- (6) Ruban, A. V.; Johnson, M. P.; Duffy, C. D. P. The Photoprotective Molecular Switch in the Photosystem II Antenna. *Biochim. Biophys. Acta, Bioenerg.* **2012**, *1817*, 167–181.
- (7) Ruban, A. V.; Berera, R.; Iliaia, C.; van Stokkum, I. H. M.; Kennis, J. T. M.; Pascal, A. A.; van Amerongen, H.; Robert, B.; Horton, P.; van Grondelle, R. Identification of a Mechanism of Photoprotective Energy Dissipation in Higher Plants. *Nature* **2007**, *450*, 575–578.
- (8) Holt, N. E.; Zigmantas, D.; Valkunas, L.; Li, X.-P.; Niyogi, K. K.; Fleming, G. R. Carotenoid Cation Formation and the Regulation of Photosynthetic Light Harvesting. *Science* **2005**, *307*, 433–436.
- (9) Ahn, T. K.; Avenson, T. J.; Ballottari, M.; Cheng, Y.-C.; Niyogi, K. K.; Bassi, R.; Fleming, G. R. Architecture of a Charge-Transfer State Regulating Light Harvesting in a Plant Antenna Protein. *Science* **2008**, *320*, 794–797.
- (10) Pascal, A. A.; Liu, Z.; Broess, K.; van Oort, B.; van Amerongen, H.; Wang, C.; Horton, P.; Robert, B.; Chang, W.; Ruban, A. Molecular Basis of Photoprotection and Control of Photosynthetic Light-Harvesting. *Nature* **2005**, *436*, 134–137.
- (11) Hudson, B. S.; Kohler, B. E. A Low-Lying Weak Transition in the Polyene α,ω -Diphenyloctatetraene. *Chem. Phys. Lett.* **1972**, *14*, 299–304.
- (12) Schulten, K.; Karplus, M. On the Origin of a Low-lying Forbidden Transition in Polyenes and Related Molecules. *Chem. Phys. Lett.* **1972**, *14*, 305–309.
- (13) Polívka, T.; Sundström, V. Ultrafast Dynamics of Carotenoid Excited States—From Solution to Natural and Artificial Systems. *Chem. Rev.* **2004**, *104*, 2021–2072.
- (14) Polívka, T.; Frank, H. J. Molecular Factors Controlling Photosynthetic Light Harvesting by Carotenoids. *Acc. Chem. Res.* **2010**, *43*, 1125–1134.
- (15) Polívka, T.; Sundström, V. Dark Excited States of Carotenoids: Consensus and Controversy. *Chem. Phys. Lett.* **2009**, *477*, 1–11.
- (16) Cerullo, G.; Polli, D.; Lanzani, G.; De Silvestri, S.; Hashimoto, H.; Cogdell, R. J. Photosynthetic Light Harvesting by Carotenoids: Detection of an Intermediate Excited State. *Science* **2002**, *298*, 2395–2398.
- (17) Ostroumov, E.; Müller, M. G.; Marian, C. M.; Kleinschmidt, M.; Holzwarth, A. R. Electronic Coherence Provides a Direct Proof for Energy-Level Crossing in Photoexcited Lutein and β -Carotene. *Phys. Rev. Lett.* **2009**, *103*, 108302.
- (18) Marek, M. S.; Backup, T.; Motzkus, M. Direct Observation of a Dark State in Lycopene Using Pump-DFWM. *J. Phys. Chem. B* **2011**, *115*, 8328–8337.
- (19) Liddell, P. A.; Kuciauskas, D.; Sumida, J. P.; Nash, B.; Nguyen, D.; Moore, A. L.; Moore, T. A.; Gust, D. Photoinduced Charge Separation and Charge Recombination to a Triplet State in a Carotene–Porphyrin–Fullerene Triad. *J. Am. Chem. Soc.* **1997**, *119*, 1400–1405.
- (20) Kodis, G.; Herrero, C.; Palacios, R.; Mariño-Ochoa, E.; Gould, S.; De La Garza, L.; Van Grondelle, R.; Gust, D.; Moore, T. A.; Moore, A. L.; et al. Light Harvesting and Photoprotective Functions of Carotenoids in Compact Artificial Photosynthetic Antenna Designs. *J. Phys. Chem. B* **2004**, *108*, 414–425.
- (21) Gust, D.; Moore, T. A.; Moore, A. L. Solar Fuels via Artificial Photosynthesis. *Acc. Chem. Res.* **2009**, *42*, 1890–1898.
- (22) Klox, M.; Pillai, S.; Kodis, G.; Gust, D.; Moore, T. A.; Moore, A. L.; van Grondelle, R.; Kennis, J. T. M. Carotenoid Photoprotection in Artificial Photosynthetic Antennas. *J. Am. Chem. Soc.* **2011**, *133*, 7007–7015.
- (23) Klox, M.; Pillai, S.; Kodis, G.; Gust, D.; Moore, T. A.; Moore, A. L.; van Grondelle, R.; Kennis, J. T. M. New Light-Harvesting Roles of Hot and Forbidden Carotenoid States in Artificial Photosynthetic Constructs. *Chem. Sci.* **2012**, *3*, 2052–2061.
- (24) Gust, D.; Moore, T. A.; Moore, A. L.; Devadoss, C.; Liddell, P. A.; Hermant, R.; Nieman, R. A.; Demanche, L. J.; Degraziano, J. M.; Gouni, I. Triplet and Singlet Energy Transfer in Carotene-Porphyrin Dyads: Role of the Linkage Bonds. *J. Am. Chem. Soc.* **1992**, *114*, 3590–3603.
- (25) Manzoni, C.; Polli, D.; Cerullo, G. Two-Color Pump-probe System Broadly Tunable over the Visible and the Near Infrared with Sub-30 fs Temporal Resolution. *Rev. Sci. Instrum.* **2006**, *77*, 023103.
- (26) Polli, D.; Luer, L.; Cerullo, G. High-Time-Resolution Pump-Probe System with Broadband Detection for the Study of Time-Domain Vibrational Dynamics. *Rev. Sci. Instrum.* **2007**, *78*, 103108.
- (27) van Stokkum, I. H. M.; Larsen, D. S.; van Grondelle, R. Global and Target Analysis of Time-Resolved Spectra. *Biochim. Biophys. Acta* **2004**, *1657*, 82–104.
- (28) Snellenburg, J. J.; Liptonok, S. P.; Seger, R.; Mullen, K. M.; van Stokkum, I. H. M. Glotaran: A Java-Based Graphical User Interface for the R Package TIMP. *J. Stat. Software* **2012**, *49*, 1–22.
- (29) de Weerd, F. L.; van Stokkum, I. H. M.; van Grondelle, R. Subpicosecond Dynamics in the Excited State Absorption of All-Trans- β -Carotene. *Chem. Phys. Lett.* **2002**, *354*, 38–43.
- (30) Polli, D.; Cerullo, G.; Lanzani, G.; De Silvestri, S.; Hashimoto, H.; Cogdell, R. J. Carotenoid-Bacteriochlorophyll Energy Transfer in LH2 Complexes Studied with 10-fs Time Resolution. *Biophys. J.* **2006**, *90*, 2486–2497.
- (31) Gradinaru, C. C.; Kennis, J. T.; Papagiannakis, E.; van Stokkum, I. H.; 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. U. S. A.* **2001**, *98*, 2364–2369.
- (32) Papagiannakis, E.; Kennis, J. T. M.; van Stokkum, I. H. M.; Cogdell, R. J.; van Grondelle, R. An Alternative Carotenoid-to-Bacteriochlorophyll Energy Transfer Pathway in Photosynthetic Light Harvesting. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 6017–6022.
- (33) Savolainen, J.; Backup, T.; Hauer, J.; Jafarpour, A.; Serrat, C.; Motzkus, M.; Herek, J. L. Carotenoid Deactivation in an Artificial Light-Harvesting Complex via a Vibrationally Hot Ground State. *Chem. Phys.* **2009**, *357*, 181–187.
- (34) 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 S1 and S* States. *J. Phys. Chem. B* **2006**, *110*, 5727–5736.
- (35) Krueger, B. P.; Scholes, G. D.; Jimenez, R.; Fleming, G. R. Electronic Excitation Transfer from Carotenoid to Bacteriochlorophyll in the Purple Bacterium *Rhodospseudomonas Acidophila*. *J. Phys. Chem. B* **1998**, *102*, 2284–2292.
- (36) Macpherson, A. N.; Arellano, J. B.; Fraser, N. J.; Cogdell, R. J.; Gillbro, T. Efficient Energy Transfer from the Carotenoid S(2) State in a Photosynthetic Light-Harvesting Complex. *Biophys. J.* **2001**, *80*, 923–930.
- (37) Wohlleben, W.; Backup, T.; Herek, J. L.; Cogdell, R. J.; Motzkus, M. Multichannel Carotenoid Deactivation in Photosynthetic Light Harvesting as Identified by an Evolutionary Target Analysis. *Biophys. J.* **2003**, *85*, 442–450.
- (38) Savolainen, J.; Dijkhuizen, N.; Fanciulli, R.; Liddell, P. A.; Gust, D.; Moore, T. A.; Moore, A. L.; Hauer, J.; Backup, T.; Motzkus, M.; et al. Ultrafast Energy Transfer Dynamics of a Bioinspired Dyad Molecule. *J. Phys. Chem. B* **2008**, *112*, 2678–2685.
- (39) Cong, H.; Niedzwiedzki, D. M.; Gibson, G. N.; LaFountain, A. M.; Kelsh, R. M.; Gardiner, A. T.; Cogdell, R. J.; Frank, H. A. Ultrafast Time-Resolved Carotenoid to-Bacteriochlorophyll Energy Transfer in LH2 Complexes from Photosynthetic Bacteria. *J. Phys. Chem. B* **2008**, *112*, 10689–10703.

- (40) Berera, R.; van Stokkum, I. H. M.; Kodis, G.; Keirstead, A. E.; Pillai, S.; Herrero, C.; Palacios, R. E.; Vengris, M.; van Grondelle, R.; Gust, D.; et al. Energy Transfer, Excited-State Deactivation, and Exciplex Formation in Artificial Caroteno-Phthalocyanine Light-Harvesting Antennas. *J. Phys. Chem. B* **2007**, *111*, 6868–6877.
- (41) Papagiannakis, E.; Das, S. K.; Gall, A.; Van Stokkum, I. H. M.; Robert, B.; van Grondelle, R.; Frank, H. A.; Kennis, J. T. M. Light Harvesting by Carotenoids Incorporated into the B850 Light-Harvesting Complex from *Rhodobacter sphaeroides* R-26.1: Excited-State Relaxation, Ultrafast Triplet Formation, and Energy Transfer to Bacteriochlorophyll. *J. Phys. Chem. B* **2003**, *107*, 5642–5649.
- (42) Wohlleben, W.; Buckup, T.; Hashimoto, H.; Cogdell, R. J.; Herek, J. L.; Motzkus, M. Pump–Deplete–Probe Spectroscopy and the Puzzle of Carotenoid Dark States. *J. Phys. Chem. B* **2004**, *108*, 3320–3325.
- (43) Rademaker, H.; Hoff, A. J.; Van Grondelle, R.; Duysens, L. N. M. Carotenoid Triplet Yields in Normal and Deuterated *Rhodospirillum rubrum*. *Biochim. Biophys. Acta, Bioenerg.* **1980**, *592*, 240–257.
- (44) Kingma, H.; van Grondelle, R.; Duysens, L. N. M. Magnetic-Field Effects in Photosynthetic Bacteria. II. Formation of Triplet States in the Reaction Center and the Antenna of *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides*. Magnetic-Field Effects. *Biochim. Biophys. Acta, Bioenerg.* **1985**, *808*, 383–399.
- (45) Niedzwiedzki, D.; Kosciulecki, J. F.; Cong, H.; O Sullivan, J.; Gibson, G. N.; Birge, R. R.; Frank, H. A. Ultrafast Dynamics and Excited State Spectra of Open-Chain Carotenoids at Room and Low Temperatures. *J. Phys. Chem. B* **2007**, *111*, 5984–5998.
- (46) Bode, S.; Quentmeier, C. C.; Liao, P.-N.; Hafi, N.; Barros, T.; Wilk, L.; Bittner, F.; Walla, P. J. On the Regulation of Photosynthesis by Excitonic Interactions Between Carotenoids and Chlorophylls. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 12311–12316.
- (47) Berera, R.; Herrero, C.; van Stokkum, I. H. M.; Vengris, M.; Kodis, G.; Palacios, R. E.; van Amerongen, H.; van Grondelle, R.; Gust, D.; Moore, T. A.; et al. A Simple Artificial Light-Harvesting Dyad as a Model for Excess Energy Dissipation in Oxygenic Photosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 5343–5348.
- (48) Frank, H. A.; Cua, A.; Chynwat, V.; Young, A.; Gosztola, D.; Wasielewski, M. R. Photophysics of the Carotenoids Associated with the Xanthophyll Cycle in Photosynthesis. *Photosynth. Res.* **1994**, *41*, 389–395.
- (49) Palacios, R. E.; Kodis, G.; Herrero, C.; Ochoa, E. M.; Gervaldo, M.; Gould, S. L.; Kennis, J. T. M.; Gust, D.; Moore, T. A.; Moore, A. L. Tetrapyrrole Singlet Excited State Quenching by Carotenoids in an Artificial Photosynthetic Antenna. *J. Phys. Chem. B* **2006**, *110*, 25411–25420.
- (50) Liao, P.-N.; Pillai, S.; Gust, D.; Moore, T. A.; Moore, A. L.; Walla, P. J. Two-Photon Study on the Electronic Interactions Between the First Excited Singlet States in Carotenoid-Tetrapyrrole Dyads. *J. Phys. Chem. A* **2011**, *115*, 4082–4091.
- (51) Engel, G. S.; Calhoun, T. R.; Read, E. L.; Ahn, T.-K.; Mancal, T.; Cheng, Y.-C.; Blankenship, R. E.; Fleming, G. R. Evidence for Wavelike Energy Transfer through Quantum Coherence in Photosynthetic Systems. *Nature* **2007**, *446*, 782–786.
- (52) Collini, E.; Wong, C. Y.; Wilk, K. E.; Curmi, P. M. G.; Brumer, P.; Scholes, G. D. Coherently Wired Light-Harvesting in Photosynthetic Marine Algae at Ambient Temperature. *Nature* **2010**, *463*, 644–647.
- (53) Calhoun, T. R.; Ginsberg, N. S.; Schlau-Cohen, G. S.; Cheng, Y.-C.; Ballottari, M.; Bassi, R.; Fleming, G. R. Quantum Coherence Enabled Determination of the Energy Landscape in Light-Harvesting Complex II. *J. Phys. Chem. B* **2009**, *113*, 16291–16295.