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Ultrabroadband two-dimensional electronic spectroscopy reveals energy flow pathways in LHCII across the visible spectrum

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Abstract. We utilise ultrabroadband two-dimensional electronic spectroscopy to map out pathways of energy flow in LHCII across the entire visible region. In addition to the well-established, low-lying chlorophyll Q_y bands, our results reveal additional pathways of energy relaxation on the higher-lying excited states involving the S_2 energy levels of carotenoids, including ultrafast carotenoid-to-chlorophyll energy transfer on 90-150 fs timescales.

1 Introduction

In early steps of photosynthesis, sunlight is captured by a densely packed network of pigment-protein complexes (PPCs) and efficiently funnelled down towards the reaction centre on an ultrafast timescale [1]. In green plants, light-harvesting complex II (LHCII, Fig. 1a), the most abundant and thus the major PPC found in plants, plays the primary light-harvesting role. LHCII serves as an efficient harvester of visible sunlight, as reflected on its broad absorption spectrum across the visible region (Fig. 1b). The broad absorption range is achieved by binding a large number of chlorophyll (Chl) and carotenoid (Car) pigments. The accessory light-harvesting pigments Cars exhibit an absorption maximum at the wavelengths where Chls poorly absorb, mediating the large energy gap between the higher-lying Soret and lower-lying Q states of Chls. However, earlier work on the pathways of energy transfer (ET) in LHCII has largely been constrained to the two lowest-energy levels of Chls [2], which corresponds to only 20% of the visible solar spectrum by area.

In this work, we uncover the full pathways of energy flow in LHCII by utilizing ultrabroadband two-dimensional electronic spectroscopy (2DES) with spectral coverage across the entire visible region. The expanded spectral bandwidth and 10 fs temporal resolution enable us to simultaneously probe the broad range of pigment excited states in LHCII and map out how the absorbed solar energy relaxes down from higher-lying states to the lowest-energy Chl Q bands, including direct ET from Car S_2 to Chl Q states.

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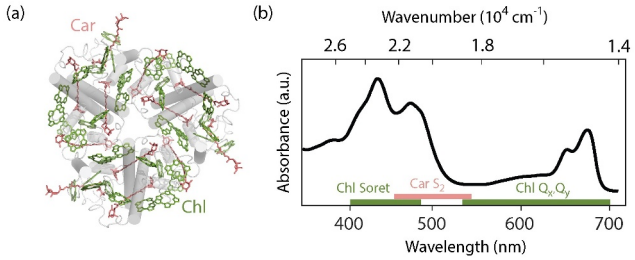

Fig. 1. (a) Structural model (top view) of a trimeric LHCII complex based on its crystal structure (PDB 1RWT). Chls are displayed in green, Cars are displayed in pink, and the protein matrix is shown in grey. (b) Linear absorption spectrum of LHCII. Coloured bars indicate the pigment transitions giving rise to the corresponding region of the spectrum.

2 Experimental details

The ultrabroadband laser spectrum was generated by focusing the output of a Ti:sapphire regenerative amplifier (Coherent Libra, 5 kHz, 1.1 mJ, ~40 fs pulse duration centred at 800 nm) into pressurised argon gas. The output of the argon chamber was spectrally filtered with glass bandpass filters, resulting in a spectral bandwidth (fwhm) of 3934 cm^{-1} (117 nm, centred at 550 nm, see Fig. 2a for the final laser spectrum employed in 2DES). The pulse was compressed with chirped mirrors into 10 fs temporal fwhm, and sent into an all-reflective 2DES setup with BOXCARS phase-matching geometry. Additional technical details on the ultrabroadband 2DES apparatus can be found in [3]. The LHCII protein was extracted from spinach and solubilised in 10 mM HEPES, 20 mM NaCl buffer containing 0.03% α-dodecylmaltoside detergent (pH = 7.5). The 2DES measurement was performed at 4°C in a 200-μm quartz flow cell.

3 Results and discussion

A representative ultrabroadband 2D spectrum of LHCII at a waiting time (T) of 600 fs is shown in Fig. 2a, revealing rich spectral features across the visible spectrum. On the upper right corner of the 2D spectrum, we see spectral signatures of ultrafast $S_2 \rightarrow S_1$ internal conversion of Cars, shown as the concomitant decay of S_2 ground-state bleach (GSB)/stimulated emission (SE) and rise of S_1 excited-state absorption (ESA) [4]. Competing with this pathway, several cross peaks corresponding to direct ET from Car S_2 to Chl Q_x/Q_y states are observed on the lower right corner (grey box in Fig. 2a). Remarkably, all of the Car-Chl cross peaks observed grow in with 90-150 fs timescales (see Fig. 2c for a representative time trace), which are much more rapid than predicted by theory considering the large energy gap of the donor (Car S_2 states) and the acceptor (Chl Q states), which can be as large as 5000 cm^{-1} [5].

As LHCII binds four different Car pigments per monomeric subunit (two luteins, one neoxanthin, and one violaxanthin under low light condition), each of which absorbs at different energies [4], we were able to resolve the contribution of the individual Cars as energy donors to the Chl acceptors by monitoring the cross peak intensities at the corresponding excitation frequencies (Fig. 2b). Notably, the Car-Chl cross peak intensity was found to be up to four times higher at the excitation frequency of the lower-energy lutein (lutein 2) compared to the higher-lying lutein (lutein 1), suggesting that lutein 2 acts as the primary donor of energy towards Chls. Being the lowest-energy one of the four Cars

in LHCII, lutein 2 reduces the donor-acceptor energy gap by ~1000 cm^{-1}, providing one of the mechanisms for the efficient and rapid Car-Chl ET observed. In contrast, lutein 1, whose S_2 state lies higher in energy than that of lutein 2 by ~800 cm^{-1}, shows little direct ET to Chls. From these results, we can infer that the two luteins may play different primary photophysical roles in plant light harvesting despite their identical chemical structure, likely due to their distinct spatial configurations imposed by the local structure of the protein binding pocket. The potentially different functions of the two luteins have indeed been proposed in earlier crystallography studies [6]: the conjugated polyene chain of lutein 1 possesses a more favourable conformation to form a photoprotective site with neighbouring Chls, qualitatively in agreement with the lower amplitude of ET towards Chls observed in our 2D spectra. While further studies are required to determine the molecular parameters behind the distinct Car functions, ultrabroadband 2DES offers new insights into how plants optimally capture and utilise the broad bandwidth of the visible sunlight.

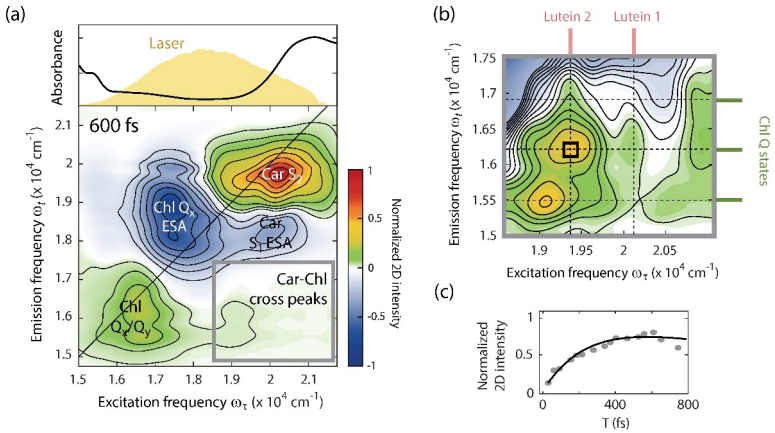

Fig. 2. (a) Absorptive 2D spectrum of LHCII at a waiting time (*T*) of 600 fs. Top panel shows the linear absorption of LHCII in the detection range of 2DES (black line) overlaid with the ultrabroadband laser spectrum (yellow area). (b) Zoom-in of Car-Chl cross peak region of the 2D spectrum (labelled with grey box in (a)). Coloured sticks indicate the pigment energy levels identified by second-derivative analysis of the linear absorption spectrum. (c) Representative waiting time trace of Car-Chl cross peaks, obtained at the peak position labelled in (b) with a black box. The exponential fit shows a 120-fs rise component.

References

1. R. E. Blankenship, *Molecular mechanisms of photosynthesis* (Blackwell Science, Malden, 2002)
2. G. S. Schlau-Cohen, T. R. Calhoun, N. S. Ginsberg, E. L. Read, M. Ballottari, R. Bassi, R. van Grondelle, G. R. Fleming, J. Phys. Chem. B, **113** (2009)
3. M. Son, S. Mosquera-Vázquez, G. S. Schlau-Cohen, Opt. Express, **25** (2017)
4. T. Polívka, V. Sundström, Chem. Rev., **104** (2004)
5. C. C. Gradinaru, I. H. M. van Stokkum, A. A. Pascal, R. van Grondelle, H. van Amerongen, J. Phys. Chem. B, **104** (2000)
6. H. Yan, P. Zhang, C. Wang, Z. Liu, W. Chang, Biochem. Biophys. Res. Commun., **355** (2007)