Supporting Information for

Photoactivation mechanism, timing of protein secondary structure dynamics and carotenoid translocation in the Orange Carotenoid Protein

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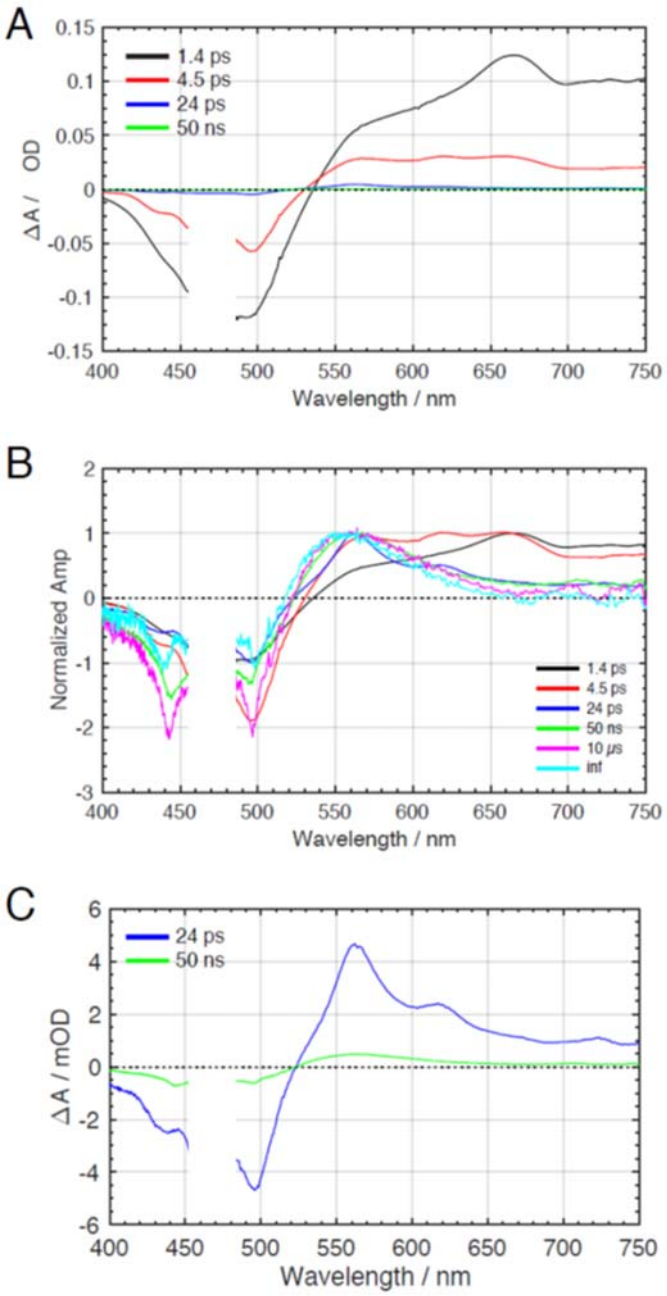

Figure S1. Fitting results following Global Analysis of UV-vis TA data. (A) EADS of the first 4 components; (B) EADS normalized to their respective absorption maxima; (C) 24 ps and 50 ns EADS, unscaled. Note the 1000-fold ordinate axis unit change between panel (A) and (C). The initially populated, short-lived S_2 state was not resolved due to sparse sampling around time zero.

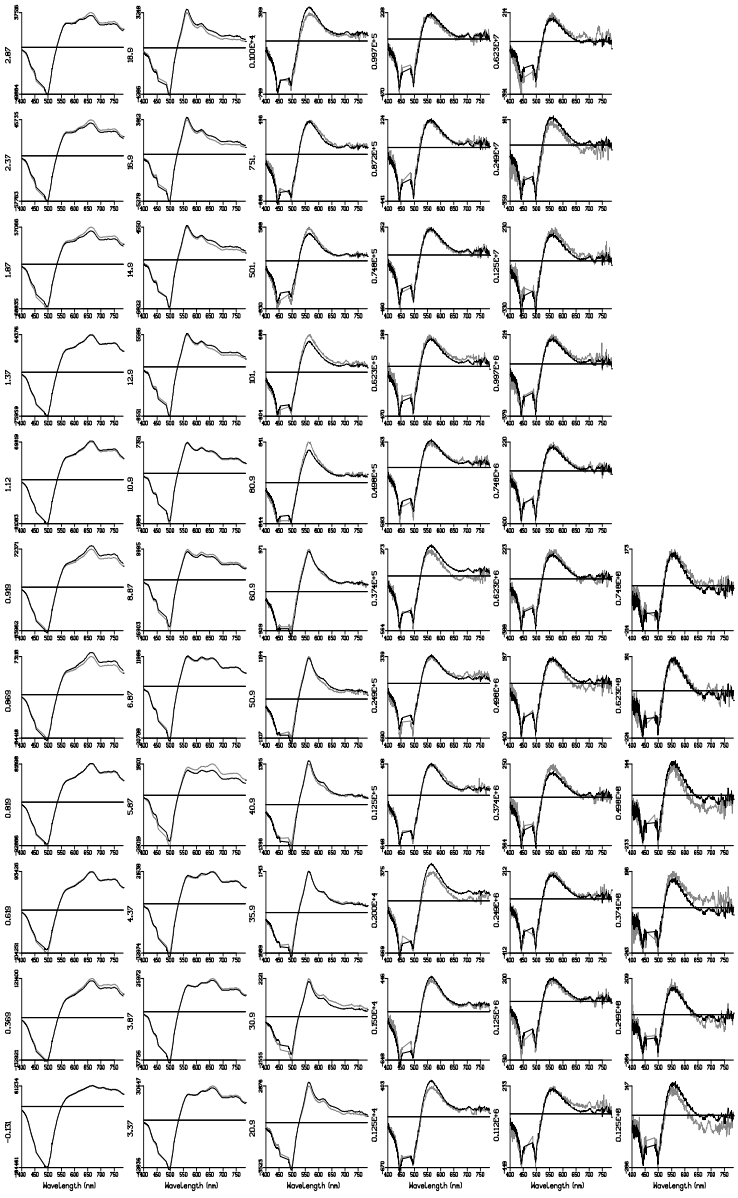

Figure S2. Time gated spectra (in μOD) at various delays (in ps, written in the ordinate labels) of UV Vis transient absorption data. Data in grey, global fit in black. The maximum of the IRF was at -0.86 ps. The last delay (top panel of right column) is 7.48×10^8 ps, which is 748 μs. Note the very large dynamic range, with minima ranging from -164461 μOD at -0.131 ps till -214 μOD at 748 μs.

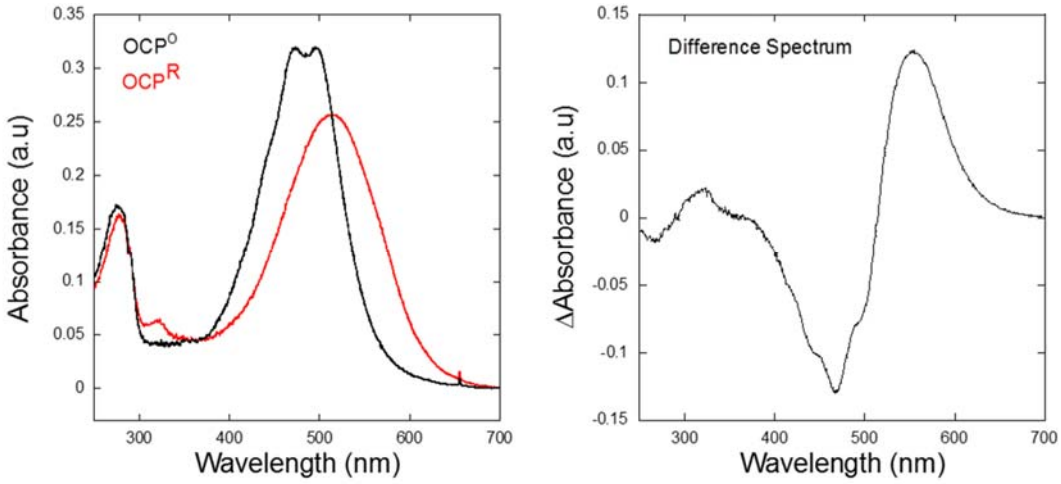

Figure S3. Absorption spectra of OCP^O and OCP^R (left panel) and difference spectrum upon full photoconversion (right panel). These spectra are used in the estimation of the quantum yield of P_1 formation. The negative amplitude (bleach) of the P_1 difference signal amounts to 0.5% of that of the S_1/ICT signal (Fig. S1A,C), which would suggest an approximate 0.5 % quantum yield. However, in the P_1 difference spectrum, there is extensive mutual compensation of the OCP^O bleach and the P_1 induced absorption. In contrast, in the S_1/ICT difference signal, there is no or little of such compensation because the S_1/ICT absorption is well separated from the OCP^O ground state bleach. Therefore, a 0.5 % quantum yield for P_1 formation would be an underestimation. To roughly estimate the extent of mutual compensation in the P_1 difference spectrum, we compare the OCP^R minus OCP^O difference spectrum with that of the OCP^O spectrum upon full photoconversion, thereby assuming that the spectral shapes of OCP^R and P_1 are sufficiently similar to make such comparison. We observe that the OCP^O bleach amplitude in the difference spectrum (right panel) amounts to approximately $1/3^{rd}$ of that of OCP^O absorption spectrum (left panel). From these considerations, the quantum yield of P_1 formation is estimated at approximately 3 x 0.5 % = 1.5 %.

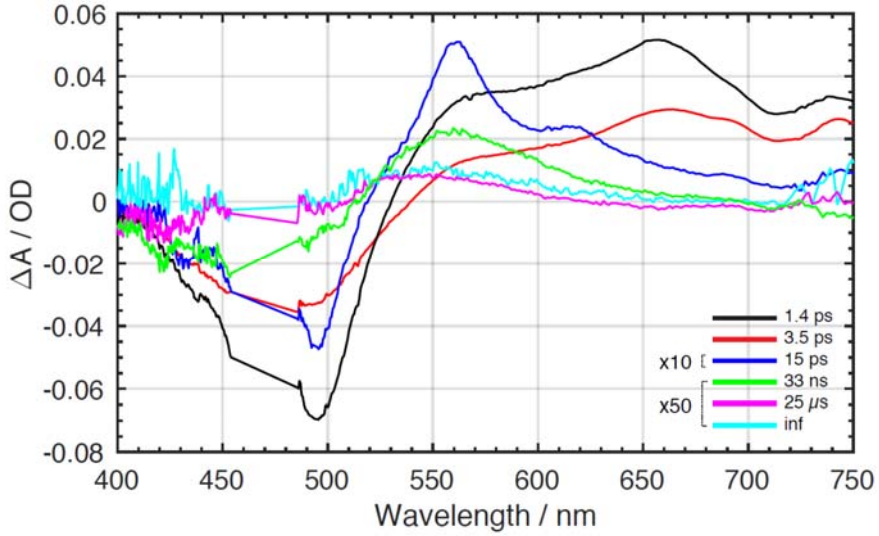

Figure S4. EADS of OCP at 475 nm excitation, at 150 nJ excitation pulse energy. The excitation pulse was stretched to 2 ps. The 15 ps EADS was expanded 10 times, while the 33 ns, 25 μs and infinite EADS were expanded 50 times.

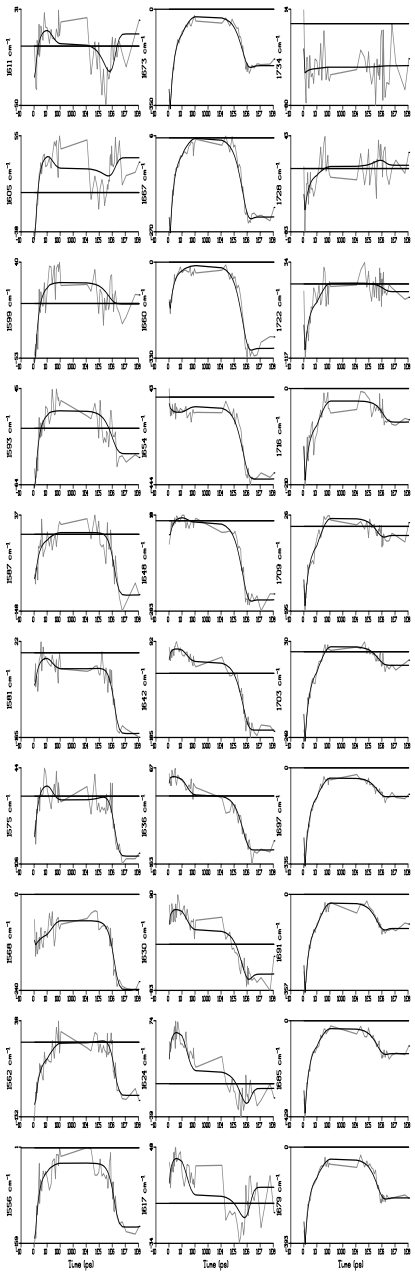

Figure S5. Kinetic traces (in μOD) at various wavenumbers (written in the ordinate label) of mid-IR transient difference absorption data. Key: data (grey), fit (black). Note that the time axis is linear until 10 ps, and logarithmic thereafter.

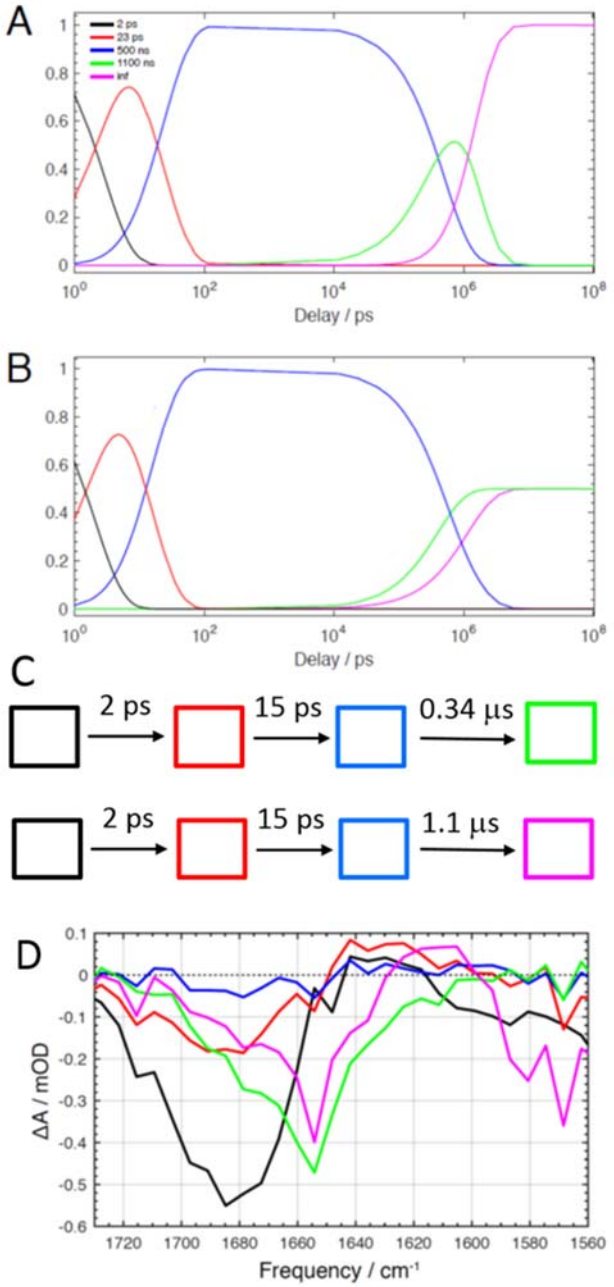

Figure S6. Transient concentration profiles of time-resolved IR data with sequential (A) and parallel (B) data analysis. Panel (C) shows the kinetic model for the parallel analysis. The black, red and blue compartments were assumed to have identical spectra. The population ratio between upper and lower pathway was fixed at 1:1. Panel (D) shows the species-associated difference spectra of the global analysis in terms of the kinetic model of (C).

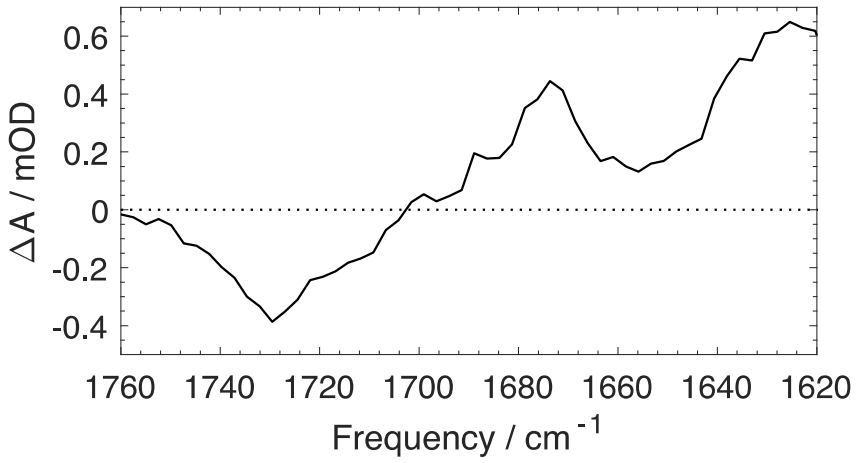

Figure S7. Mid-IR transient absorption spectrum of hECN dissolved in THF at a 2 ps delay. The excitation wavelength was 475 nm.

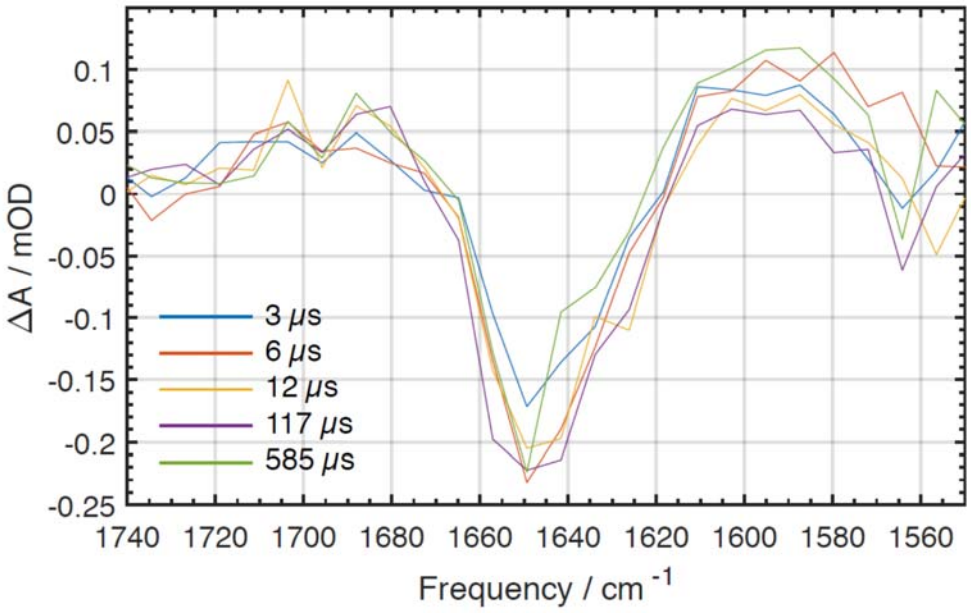

Figure S8. Mid-IR transient absorption spectrum of OCP at time delays indicated.