Ultrafast Energy Flow and Equilibration Dynamics in Photosynthetic Light-Harvesting Complexes

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Abstract: We disentangle various energy transfer pathways in the bacterio-chlorophyll excitation cascade from LH2 to LH1 in *Chromatium vinosum* grown under high-light or low-light illumination using tunable narrowband selective excitation and broadband infrared probing.

Purple bacteria are excellent model organisms to investigate the basic light-harvesting (LH) mechanisms in Nature because of their relative simplicity and the availability of high-resolution X-ray crystallographic structures [1]. Their photosynthetic unit exhibits a quasi-2D architecture made up of circular LH pigment-protein complexes: the core LH1-reaction center (RC) complex, where charge separation occurs, is surrounded by several peripheral LH2 complexes. Through a cascading effect, the absorbed energy is efficiently transferred from high to low photon-energy complexes towards the RC.

In this work we investigate the energy transfer (ET) pathways in the photosynthetic membranes of *Chromatium vinosum*, grown either under high-light (HL) or low-light (LL) conditions, using pump-probe spectroscopy in the near-infrared (NIR) region [2]. In these bacteria, illumination intensity during growth strongly affects the type of LH2 complexes synthesized, their optical spectra, and their amount of energetic disorder. We designed a specially tailored pump-probe apparatus for this study [2]. To provide selective excitation of each BChl species in such spectrally congested samples we generated narrowband (≈5nm) pump pulses, tunable from 1.57 to 1.30 eV photon energy, by an optical parametric amplifier. Broadband probe pulses in the NIR spectral region were generated via supercontinuum in a sapphire plate and detected using a single-shot low-noise spectrometer.

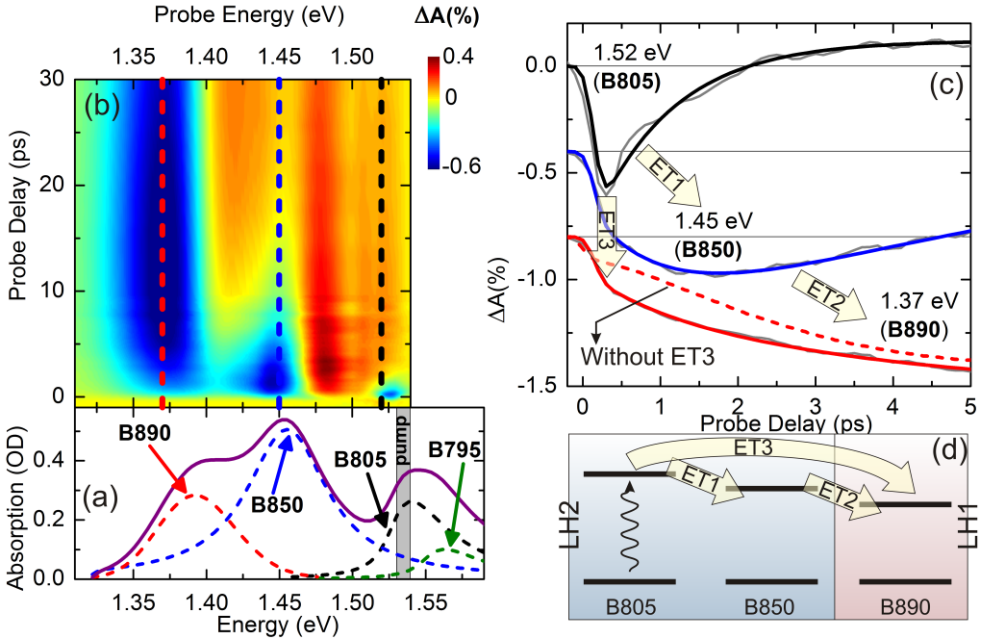

Fig. 1. (a) Ground-state absorption spectrum of THL40 and its decomposition into BChl bands fitted using Voigt lineshapes. (b) Transient absorption map of THL40 upon excitation at 1.53 eV. (c) Measured (gray thin lines) and fitted (coloured thick lines) pump-probe dynamics at selected probe photon energies. Dashed red line is a simulated curve considering only ET1 and ET2 channels active. (d) Level scheme indicating the ET processes.

Figure 1(a) shows the absorption spectrum of the THL40 sample, grown under HL illumination. We observe the presence of three different Bacterio-Chlorophylls (BChls) in the LH2 complexes, named B795, B805 and B850 due to their ground state absorption, while the LH1 complex only contains B890 BChls. Figure 1(b) shows the measured two-dimensional transient absorption (ΔA) map for the THL40 sample as a function of probe photon energy and delay after excitation at 1.53 eV, thus predominantly pumping the B805 BChls (see also grey bar in Fig. 1(a)). The pump-probe map can be decomposed into three

contributions from the B805, B850 and B890 BChls, each providing a transient spectrum made of a negative exciton photobleaching (PB) signal (blue color code) and a positive photo-induced absorption (PA) signal (red color code) from the exciton to the bi-exciton state, slightly blue-shifted in energy with respect to the corresponding PB. Around time zero the predominant contribution is from B805. Within LH2 complexes a first ET process (ET1) occurs from B805 to B850, responsible for the rise of the signal at 1.45 eV completed within ≈2ps. This is also clear looking at the pump-probe traces at selected probe energies plotted in Fig. 1(c). Subsequent ET from the B850 in LH2 towards the B890 in LH1 (called ET2) occurs in a few ps and is responsible for the delayed formation of the signal at 1.37 eV (red line in Fig. 1(c)). The B890 relaxes back to the ground state in hundreds of ps (not shown here). We performed global analysis of the data (see the fits as thick solid lines in Fig. 1(c)): the comparison of the extracted decay-associated spectra clearly confirmed the existence of the ET3 mechanism.

Looking more deeply into the early dynamics of Fig. 1(c), we note two further very important details: (i) The transient signals at 1.45 and 1.37 eV (blue and red curves) show a partial instantaneous formation (within the pump pulse) due to the fact that at 1.53-eV pump photon energy there is also a partial excitation of the B850 and B890 BChls (see Fig. 1(a)). (ii) After this instantaneous formation, the signal at 1.37 eV (red line in Fig. 1(c)), which purely monitors the dynamics of the B890 moiety, further rises with a slope which is steepest at early delays (i.e. the slope reduces in time and reaches a plateau around 15-ps delay). This demonstrates that a direct, one-step B805 (in LH2) → B890 (in LH1) ET is also active in the THL40 sample (named ET3), parallel to the sequential B800→B850→B890 two-step ET1 and ET2 processes. If this was not the case, the dynamics would look different: see for comparison the simulated time trace (red dashed line in Fig. 1(c)), showing an inflection point at early delays. A scheme of the energy levels and ET processes is sketched in Fig. 1(d).

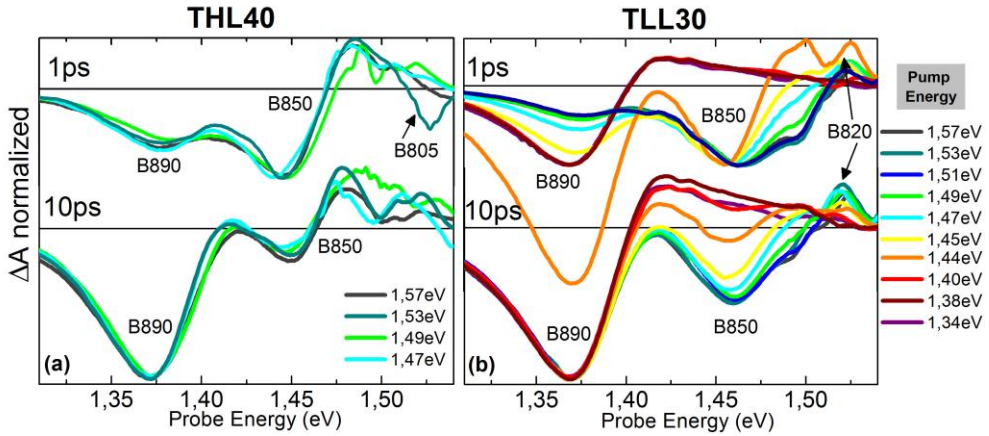

Fig. 2. Transient absorption spectra for THL40 (a) and TLL30 (b) samples at various pump photon energies at selected delays: 1ps (top panels, normalized for the B850 signal) and 10ps (bottom panels, normalized for the B890 signal)

We performed a thorough study of the energy flow and equilibration among LH2 and LH1 as a function of pump photon energy and illumination condition during growth. Transient absorption spectra at selected delays (1ps, top panel, 10ps, bottom panel) for various excitation energies are reported for the THL40 sample (Fig. 2(a)) and for the TLL30 sample (Fig. 2(b)), which was grown under LL illumination and contains an extra BChl in the LH2 called B820. In THL40 (Fig. 2a), the spectral shapes are weakly dependent on the pump energy. At 1ps delay we can see the sharp PB bands of B805 in THL40, peaked at 1.53eV, which is not present in TLL30 due to the accelerated rate caused by the additional B800→B820 ET. In TLL30 (Fig. 2(b)) on the contrary we observe a strong dependence on pump excitation: (i) at B850 (1.45eV) and B890 (1,40 eV) no B820 is found at 1ps and 10ps delays, suggesting that no significant back transfer from B850 nor B890 occurs towards B820. (ii) Upon B805 excitation at 1.53eV, we observe a very strong B820 feature at 1ps delay, indicating efficient B805→B820 ET. (iii) In all the transient spectra with a B820 signature, the B820/B850 ratio at 10ps delay is small but not vanishing: the low B820→B850 ET rate interestingly suggests that the B820 and B850 BChls reside in different LH2 complexes.

Our study sheds new light onto the selective advantage for the *Chromatium vinosum* bacterium to synthetize different LH2 complexes under HL/LL growth conditions. We are now performing two-dimensional electronic spectroscopy experiments on these samples, which will allow to disentangle the congested spectra and track the forward and back transfers.

[1] R.J. Cogdell, A. Gall, J. Köhler, "The architecture and function of the light-harvesting apparatus of purple bacteria: From single molecule to in vivo membranes" Q. Rev. Biophys. **39**, 227-324 (2006).
[2] L. Lüer *et al.*, "Tracking energy transfer between light harvesting complex 2 and 1 in photosynthetic membranes grown under high and low illumination" Proc. Natl. Acad. Sci. USA **109**, 1473-1478 (2012).