Supporting Material for manuscript

Single-Molecule Spectroscopy Unmasks the Lowest Exciton State of the B850 Assembly in LH2 from *Rps. acidophila*

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SUPPORTING MATERIAL

1. Selection of a single complex for spectroscopy

Usually the selection of a single LH complex for spectroscopy takes place in two steps. First a 40 × 40 μm^2 region of the sample is excited around 855 nm with a laser and the red-shifted fluorescence from the individual complexes located in that area is registered with a CCD camera. Subsequently the optics is switched to the confocal mode such that the excitation volume coincides with one of the complexes observed with the CCD camera. The general problem that arises with this protocol is illustrated on the example of the ensemble fluorescence-excitation spectrum, Fig. S1 *A*, *B* black line, and the emission spectrum, Fig. S1 *A*, *B* grey line, of LH2 from *Rps. acidophila*. Emission from the Ti:Sa crystal in the infrared spectral region that propagates towards the detector is suppressed by an excitation filter, the transmission of which is shown by the black dashed line in Fig. S1 *A*, *B*. The fluorescence from the sample is detected through bandpass filter-sets, and the respective transmission curves are shown by the coloured areas in Fig. S1 *A*, *B*. The wide-field images from the same area of the sample that have been registered with the two different detection filters are shown in Fig. S1 *C*, *D*. At the bottom of the wide-field images the spotted LH2 complexes are shown schematically by the coloured dots, where the colour of each dot refers to a LH2 complex that has been identified with the corresponding detection filter. Owing to the better spectral coverage of the transmission characteristics of the blue detection filter with the emission spectrum from a LH2 ensemble, the number of complexes that can be identified is larger for this filter. Hence, the choice of the detection filter plays a crucial role for the selection of a single complex for spectroscopy, because some of the complexes might not be observable while others appear brighter/dimmer as a function of the detection filter.

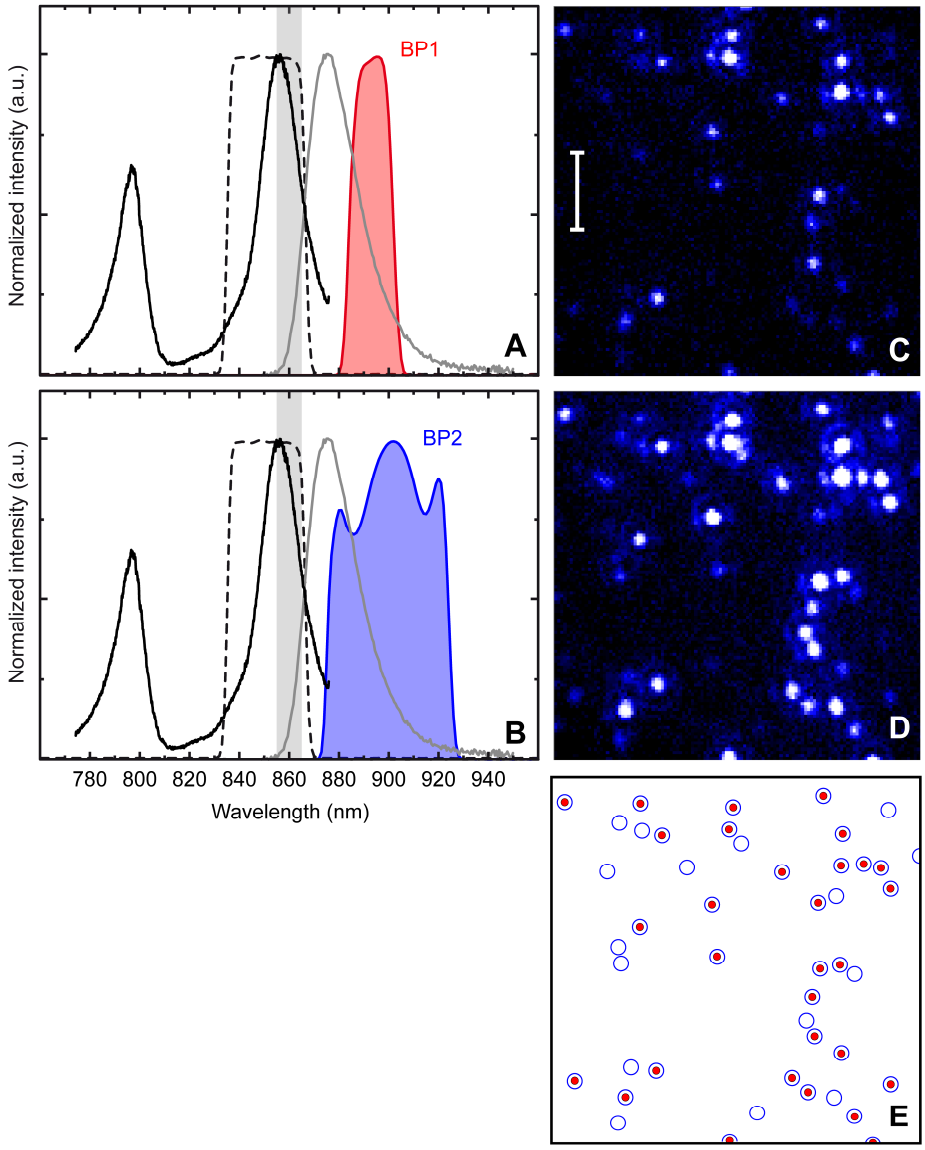

FIGURE S1 Left: Illustration of the influence of the spectral characteristics of the optical filters on the wide field imaging. Transmission curves of the detection filters with respect to the spectral positions of the absorption (*solid black lines*) and emission (*solid grey line*) of LH2 for two different detection filters. (*A*) BP1: center wavelength 893 nm, bandwidth 18 nm; (*B*) BP2: 900 nm, 48 nm. The *dashed line* corresponds to the transmission characteristics of a bandpass filter (850 nm, 30 nm) that is used in the excitation path to suppress background from the laser. All transmission curves of the optical filters are shown on a normalized scale. The sample is excited with circularly polarized light, the wavelength is wobbled between 855 nm and 865 nm (*grey shaded area*), and the excitation intensity is 100 W/cm^2. Right: Wide field images from the same sample area: (*C*) detected with filter BP1, (*D*) detected with filter BP2. The scale bar in (*C*) corresponds to 10 µm. (*E*) Schematic representation of the individual complexes that are identified in the two images. *Red dots* refer to part (*C*) of the figure (BP1), and *blue circles* refer to part (*D*) of the figure (BP2).