Supporting Information for

Fluorescence-Excitation Spectra from Individual Chlorosomes of the

Green Sulphur Bacterium *Chlorobaculum tepidum*

Marc Jendrny,† Thijs J. Aartsma,‡ and Jürgen Köhler†*

†Experimental Physics IV and Bayreuth Institute of Macromolecular Research (BIMF),
Universität Bayreuth, Universitätsstr. 30, 95447 Bayreuth, Germany

‡Leiden Institute of Physics, Leiden University, Niels Bohrweg 2, 2333 CA Leiden, The
Netherlands
ADDITIONAL EXAMPLES OF SINGLE CHLOROSOME SPECTRA

More examples of fluorescence-excitation spectra recorded from single chlorosomes at room temperature are displayed in Fig.S1.

Fig.S1: a)-c) Examples of fluorescence-excitation spectra from individual chlorosomes recorded at room temperature. The black line corresponds to the average of 70 individual fluorescence-excitation spectra and the coloured lines correspond to two individual laser scans recorded for distinct polarization angles a) 144° (red), 48° (blue); b) 156° (red), 84° (blue); c) 192° (red), 120° (blue). For better visibility the line within each spectrum corresponds to a gliding average over 11 data points of the respective spectrum.

The black line in each of the spectra corresponds to the average over all polarizations, and the
blue and red lines represent individual fluorescence-excitation spectra recorded for a distinct polarization of the excitation light. The peak positions (FWHM) of the bands are (black/red/blue): a) 13687 cm\(^{-1}\) (678 cm\(^{-1}\)) / 13663 cm\(^{-1}\) (773 cm\(^{-1}\)) / 13763 cm\(^{-1}\) (778 cm\(^{-1}\)); b) 13675 cm\(^{-1}\) (695 cm\(^{-1}\)) / 13675 cm\(^{-1}\) (673 cm\(^{-1}\)) / 13750 cm\(^{-1}\) (673 cm\(^{-1}\)); c) 13588 cm\(^{-1}\) (633 cm\(^{-1}\)) / 13575 cm\(^{-1}\) (612 cm\(^{-1}\)) / 13638 cm\(^{-1}\) (605 cm\(^{-1}\)).

Additional fluorescence-excitation spectra recorded from single chlorosomes at 1.5 K are displayed in Fig.S2. Examples of spectra that could be decomposed into two distinct spectral components due to their polarization properties are shown in Fig.S2a-c spectra, whereas Fig.S2d-f displays examples of spectra that could not be decomposed into distinct spectral components.
Fig. S2: a)-f) Examples of fluorescence-excitation spectra from individual chlorosomes recorded at 1.5 K. The black line corresponds to the average of 250 individual fluorescence-excitation spectra and the coloured lines correspond to two individual laser scans recorded for distinct polarization angles a) 240° (red), 165° (blue); b) 120° (red), 39° (blue); c) 306° (red), 231° (blue); d) 279° (red), 198° (blue); e) 177° (red), 93° (blue); f) 240° (red), 165° (blue). For better visibility the line within each spectrum corresponds to a gliding average over 11 data points of the respective spectrum.

The black line in each of the spectra corresponds to the average over all polarizations, and the
blue and red lines represent individual fluorescence-excitation spectra recorded for a distinct polarization of the excitation light. The peak positions (FWHM) of the bands are (black/red/blue): a) 13605 cm\(^{-1}\) (756 cm\(^{-1}\)) / 13354 cm\(^{-1}\) (824 cm\(^{-1}\)) / 13564 cm\(^{-1}\) (594 cm\(^{-1}\)); b) 13462 cm\(^{-1}\) (675 cm\(^{-1}\)) / 13340 cm\(^{-1}\) (670 cm\(^{-1}\)) / 13534 cm\(^{-1}\) (729 cm\(^{-1}\)); c) 13586 cm\(^{-1}\) (621 cm\(^{-1}\)) / 13462 cm\(^{-1}\) (724 cm\(^{-1}\)) / 13637 cm\(^{-1}\) (624 cm\(^{-1}\)); d) 13443 cm\(^{-1}\) (554 cm\(^{-1}\)) / 13443 cm\(^{-1}\) (540 cm\(^{-1}\)) / 13470 cm\(^{-1}\) (535 cm\(^{-1}\)); e) 13405 cm\(^{-1}\) (635 cm\(^{-1}\)) / 13351 cm\(^{-1}\) (656 cm\(^{-1}\)) / 13410 cm\(^{-1}\) (664 cm\(^{-1}\)); f) 13621 cm\(^{-1}\) (724 cm\(^{-1}\)) / 13586 cm\(^{-1}\) (702 cm\(^{-1}\)) / 13634 cm\(^{-1}\) (699 cm\(^{-1}\)).

SPAN OF THE MODULATION RATIO

Fig. S3 shows the span of the modulation ratio \(M\) for an individual chlorosome above an excitation energy of 13320 cm\(^{-1}\) for the room temperature experiments. In general, the overall distribution of the bars as well as the variations of \(M\) for an individual chlorosome are clearly narrower with respect to the distribution / variations at low temperature (see fig.3c).

![Graph showing the variation of the modulation ratio M for 24 individual chlorosomes studied at room temperature. The bars represent the minimum / maximum value of M for an individual chlorosome above a photon energy of 13320 cm\(^{-1}\).]
SAMPLE PREPARATION

Cells harvested from *Chlorobaculum (Cb.) tepidum* wild type, strain TLS (ATCC 49652) were incubated with lysozyme and then disrupted by passes through a French Press. The cell homogenate was clarified by low-speed centrifugation and a chlorosome-enriched fraction was separated from the soluble proteins by ultracentrifugation. The resulting pellet was resuspended in buffer (50 mM Tris/HCl, 10 mM Na-Ascobat, pH 8.3 at room temperature) and further purified on a 20 to 50 % continuous sucrose gradient from which two dark green bacteriochlorophyll (BChl) c containing fractions were collected (lower and upper band). The chlorosomes from both bands were diluted with buffer and two times centrifuged. The final pellet consisted of a loose fraction (designated light chlorosomes) on top of a firm pellet (designated heavy chlorosomes). For the experiments described here we used the light chlorosomes from the upper band. For experiments on ensembles the stock solution was diluted to an optical density of 0.1 (OD 0.1) at 733 nm. For single molecule experiments the stock solution of \(1.1 \times 10^{-3}\) M concentration was diluted to \(5 \times 10^{-9}\) M with buffer. A drop from this solution (about 10 µl) were adsorbed on a SiO\(_2\) glass substrate under nitrogen atmosphere for 30 min.

OPTICAL SETUP

For the optical experiments on single chlorosomes the samples were illuminated with a continuous-wave tuneable Titanium-Sapphire (Ti:Sa) laser (Coherent 899-01) pumped by a frequency doubled continuous-wave Neodymium-Yttrium-Vanadat (Nd:YVO\(_4\)) laser (Coherent Verdi V10) using a home build microscope that can be operated either in widefield or confocal mode. To obtain a well-defined variation of the wavelength of the Ti:Sa laser the intracavity birefringent filter has been rotated with a motorised micrometer screw (Melles
Griot Nanomover). For calibration purposes a wavemeter (WaveMaster, Coherent) has been used and an accuracy as well as a reproducibility of 1 cm\(^{-1}\) for the laser frequency has been verified.

First a 45 × 45 µm\(^2\) wide-field image of the sample was taken by exciting the sample around 735 nm. The emission from the sample was collected by a microscope objective (Mikrothek, NA = 0.85) that was mounted inside the cryostat and the signal was focussed onto a CCD camera (Andor LUCA\(^{EM}\) R 604) after passing band-pass filters (BP 850/80; AHF Analysetechnik) that block residual laser light. Next, a spatially well-isolated chlorosome was selected from the wide-field image, and the microscope was switched to the confocal mode. Fluorescence-excitation spectra have been recorded by scanning the linearly polarised excitation light between 715 nm and 795 nm with a scanning rate of 2.8 nm/s (≈ 47 cm\(^{-1}\)/s). The excitation intensity was about 3 W/cm\(^2\). The polarisation of the excitation light was rotated in steps of 3° between two successive scans by means of a waveplate. The emission was band-pass filtered (BP850/80; AHF Analysetechnik) and focussed onto a single-photon counting avalanche photodiode (APD) (SPCM-AQR-15, Perkin Elmer). All experiments were performed at 1.5 K. For reference experiments on ensembles of chlorosomes the excitation light was focused onto the sample with a lens (f = 100 mm) in front of the cryostat.

For room-temperature experiments on ensembles of chlorosomes we used a conventional absorption spectrometer (Perkin Elmer, Lambda 750 UV/VIS Spectrometer).