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Supporting Information

Combined Topographic, Spectroscopic, and Model Analyses of Inhomogeneous Energetic Coupling of Linear Light Harvesting Complex II Aggregates in Native Photosynthetic Membrane

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S1. Bacterial Growth and Purification:

The bacterial cells were grown with purging a gas mixture of 95% N_2 and 5% CO_2 and harvested when the absorption reach 0.5 O.D. at 600 nm ($A_{600\ nm}$). Membrane fragment vesicles were separated on a Sepharose 2B column (50 × 2.0 cm) and then purifies by rate-zonal sucrose gradient centrifugation at 63500 × g for 10 hours. Membranes were isolated by ultracentrifugation at 260000 × g for 1 hour. The isolated membranes were dissolved in 20 mM Tris-HCl buffer at pH 8.0 containing 100 mM NaCl and 1% Lauryl *N*,*N*-dimethylamine-*N*-oxide (LDAO) for the isolation of LH2 complexes by DEAE-52 cellulose chromatography and 20-40% sucrose gradient centrifugation at 260000 × g for 16 hours at 4 °C. Purified membrane fragment vesicles and LH2 complexes were stored at -80 °C.

S2. Sample Preparation and AFM Imaging:

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The photosynthetic membrane fragments were adsorbed on a freshly cleaved mica surface (Ted Pella, Inc., CA) for AFM imaging. The surface of the mica was first rinsed with 25 mM $MgCl_2$ solution to change the surface charge from negative to positive to ensure the firm attachment of the membrane fragments on the mica surface. A drop of adsorption buffer (10 mM Tris-HCl at pH 7.5, 150 mM KCl, 25 mM $MgCl_2$) was applied on the mica surface and then 2 µL of purified membrane solution was injected into it. After 1-1.5 hours incubation, the surface was gently rinsed with a buffer (10 mM Tris-HCl at pH 7.5, 150 mM KCl) to remove multilayer membrane patches, if exist, from the surface of the mica.

We used a closed-loop multipurpose AFM scanner (Agilent 5500 SPM microscope, Agilent Technologies) and an ultra sharp AFM silicon tip (Mikromasch) having a 0.6 Nm^{-1} spring constant and ~75 kHz resonant frequency to record tapping-mode AFM images. All of the images were recorded in ambient condition at 1-2 Hz line scanning frequency with 512 × 512 $pixels^2$ resolution. We compared the unchanged feathers of the same imaging area of same membrane sample in the consecutive images to ensure the non-modification by scanning operation.

S3. Confocal Fluorescence Microscopy:

The membrane fragments and purified LH2 samples were excited using 795 nm pulse laser (100 fs pulses at a repetition rate of 76 MHz, Ti:sapphire laser system, Mira 900, Coherent) with average incident power of 3-4 µW. The fluorescence images of LH2 were recorded with an inverted confocal microscope (Axiovert-200, Zeiss). A dichroic

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mirror (815 dclp, Chroma Technology) was used to direct the laser beam to the sample via a high numerical aperture objective (1.3 NA, 63×, oil immersion, Zeiss). The sample was spin-coated on a microscope coverslip (0.17 mm thickness, 18 × 18 mm^2 size, Gold Seal), and raster scanned with respect to the laser focus by a x-y electropiezo closed-loop scanning stage (H100, Mad City Lab) for imaging. The emitted fluorescence was collected by the same objective before filtering from a long pass filter (HQ825LP, Chroma Technology). For both imaging and lifetime measurements, two Si avalanche photodiode single photon counting modules were used (SPCM-AQR-14, PerkinElmer and MPD, Micro Photon Devices) as the detectors. The fluorescence images (100×100 $pixels^2$) acquired by continuous raster scanning the sample at the rate of 4 ms/pixel.

S4. Ensemble Average Spectroscopic Measurements:

A VARIAN Cary 50 Scan UV-Visible Spectrophotometer and a Quantamaster NIR Fluorometer (Photon Technology International) were used to record the ensemble-averaged absorption and emission spectra, respectively.

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Additional AFM images to show the linearly aggregated light harvesting complex II (LH2) proteins on the photosynthetic membrane

These images are recorded following the similar experimental procedure and under the same experimental conditions described in S2.

Figure S1: AFM images of light harvesting complex II in photosynthetic membranes from *Rhodobacter sphaeroides (Strain 2.4.1)*

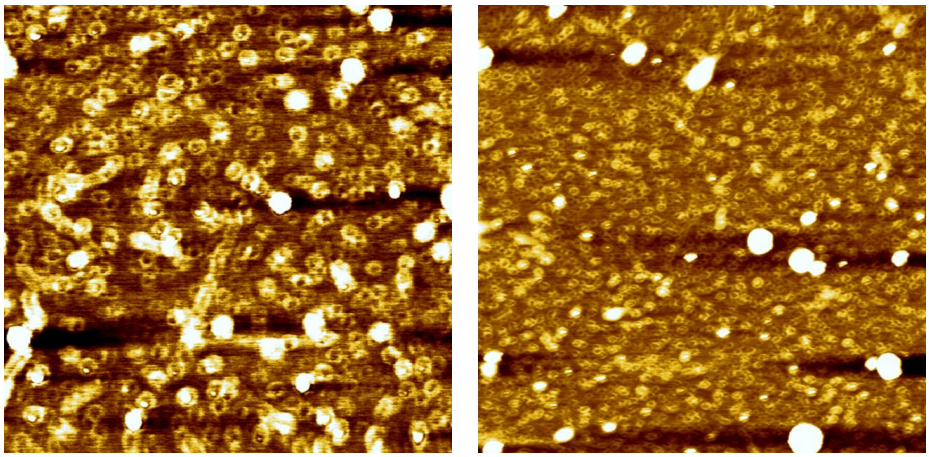

Figure S2: AFM images of light harvesting complex II in photosynthetic membranes from *Rhodobacter sphaeroides (Strain ATCC17025)*

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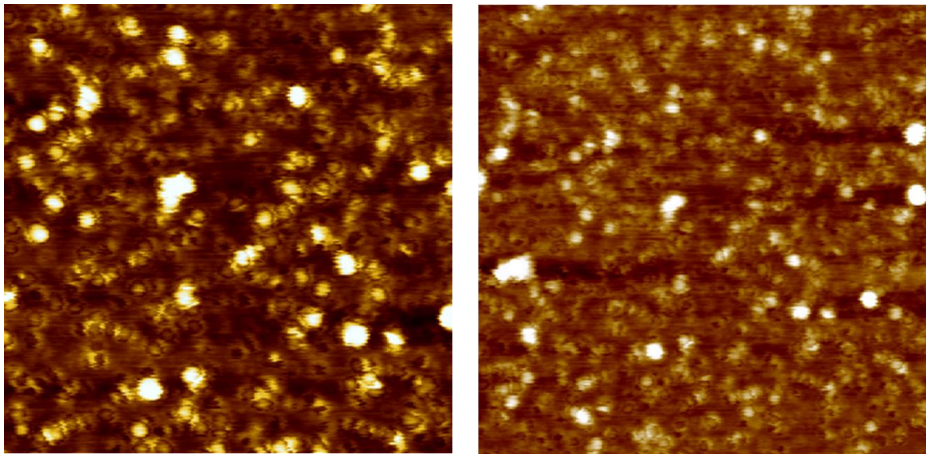

Figure S3: AFM images of light harvesting complex II in photosynthetic membranes from *Rhodospirillum fulvum.*