

Supporting Information

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SI Text

SI Methods

Sample Characterization. The sample was characterized in 50 mM PBS (pH 7.4) with 0.1% *n*-dodecyl- β -D-maltoside (DDM) by ensemble and single-molecule experiments. Ensemble time correlated single-photon counting was performed in two buffers, and both cases produced a single-component lifetime close to the values reported in the literature. The decay curves and fits are shown in Fig. S3A. The solubility and lack of aggregation of light harvesting complex 2 (LH2) in this buffer were confirmed by confocal surface-immobilized experiments and fluorescence correlation spectroscopy (FCS), as shown in Fig. S3B and C, respectively. In Fig. S3B, a confocal fluorescence intensity trace from a single LH2 is shown. The trace exhibits single-step digital blinking and photobleaching, which are hallmarks of single emitters. In Fig. S3C, a single-component fit is shown, yielding a diffusion coefficient of $37 \pm 5 \mu\text{m}^2/\text{s}$, as expected.

Data Analysis. All data analysis was performed in MATLAB (MathWorks). The time-tagged photon stream was binned into 10-ms bins to create a time trace of the intensity. The scanning pattern of the pulsed laser beam produces a time-averaged excitation profile uniform throughout the observation region on timescales longer than the ~ 0.1 -ms scanning time. On these timescales, changes in both fluorescence intensity and lifetime report on the individual complexes. To identify digital steps between intensity levels, the change-point algorithm (1) was applied to the time trace of the intensity.

To calculate fluorescence lifetime for each intensity level, a maximum likelihood estimation algorithm was used. The photons for each intensity level as determined by the change-point algorithm were binned and fit. The background level and background fluorescence decay profile was determined by hand selecting ~ 5 s of background photons from each 600-s interval of data. The background decay component was added to a decay function convolved with a measured instrument response function (IRF) with a color-shift delay introduced to match the measured IRF to the experimental data. The decay time and the color-shift delay were optimized by maximizing the log likelihood (2). Representative fits for each state are shown in Fig. S4A–C. The 95% confidence intervals were calculated by bootstrapping several hundred intensity levels, as shown in Fig. S4D.

The emission spectra from the camera were constructed by binning all the photons for a given intensity level, subtracting the background spectrum, and fitting the resulting curve to an asymmetric Gaussian using a nonlinear least-squares optimization. The background spectrum for each 100-ms frame was determined by averaging the spectra for all background intervals.

Fluorescence Lifetime and Quantum Yield. The observed fluorescence lifetime depends on the radiative rate relative to the rates of other molecular processes. The relationship between observed

fluorescence lifetime and intensity I for absorbance A is given by (3)

$$AI \propto \Phi = \frac{\tau_{\text{rad}}^{-1}}{\tau_{\text{rad}}^{-1} + \tau_{\text{quench}}^{-1}} = \frac{\tau_{\text{rad}}^{-1}}{\tau_{\text{obs}}^{-1}}$$

Assuming a constant absorbance, direct proportionality between intensity and observed lifetime arises most straightforwardly in the case in which the radiative rate is constant and the quenching rate is changing.

Theoretical Modeling

The site-basis Hamiltonian for the B850 ring was constructed by taking previously published site energies (diagonal elements) (4) and couplings (off-diagonal elements) (5). Only nearest-neighbor couplings were included for simplicity. The direction of the site-basis transition dipole moments were taken to be from N_B to N_D , which are two of the nitrogen atoms across the porphyrin ring of the bacteriochlorophylls (BChl) (6). The excited states are the energy eigenstates, or excitons, and are calculated by diagonalizing the site-basis Hamiltonian. The excited-state transition dipole moments were determined by applying the diagonalization matrix to the site-basis transition dipole moments. The simulated fluorescence spectra, $f(\omega)$, were determined by taking the three lowest excited states, thermally populating them, and dressing them with a Gaussian homogeneous line width of 100 cm^{-1} , as follows:

$$f(\omega) = \rho_1 d_1^2 e^{-\frac{(\hbar\omega - E_1)^2}{2\gamma^2}} + \rho_1 e^{-\frac{(E_2 - E_1)}{kT}} d_2^2 e^{-\frac{(\hbar\omega - E_2)^2}{2\gamma^2}} + \rho_1 e^{-\frac{(E_3 - E_1)}{kT}} d_3^2 e^{-\frac{(\hbar\omega - E_3)^2}{2\gamma^2}}$$

The delocalization length of the primary emissive state (the lowest energy state) was found by the inverse participation ratio for excited state k , given by (4)

$$P_k = \frac{1}{\sum_{n=1}^N c_n^4}$$

where c_n are the site-basis coefficients that describe their contribution to the excited states.

The photobleaching event was modeled by finding site n , where n is the site with the largest contribution to the lowest-energy exciton, setting all elements of row n and column n to zero, and re-diagonalizing the matrix. This was calculated for 1,000 realizations of static disorder, with diagonal elements of the Hamiltonian sampled from a Gaussian distribution with $\sigma_{\text{inhomogeneous}} = 200 \text{ cm}^{-1}$. The introduction of a photobleached pigment produced <1 -nm spectral shift and a small decrease in the delocalization length (inverse participation ratio) of the lowest-energy excited state.

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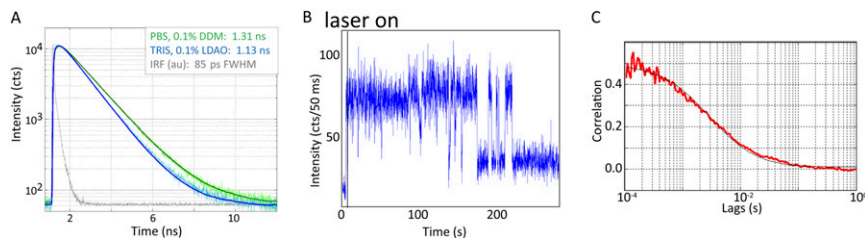


Fig. S3. Sample characterization measurements. (A) Ensemble fluorescence lifetime decays of LH2 solubilized in PBS (pH 7.4), 0.1% DDM (green) and in Tris (pH 8.0), 0.1% lauryldimethylamine oxide (blue). The instrument response function is shown in gray. The two detergents give rise to similar excited-state lifetimes, and both show single exponential fits. Experiments show no aggregation of LH2 in PBS/DDM detergent. (B) Intensity trace from a single LH2 complex immobilized in polyvinyl alcohol film, showing digital blinking and photobleaching, which suggest a single emitter. (C) Autocorrelation curve from FCS of LH2 solubilized in PBS (pH 7.4), 0.1% DDM. The autocorrelation curve shows no evidence of aggregation or multiple components, and a single component fit produces a diffusion coefficient of $37 \pm 5 \mu\text{m}^2/\text{s}$.

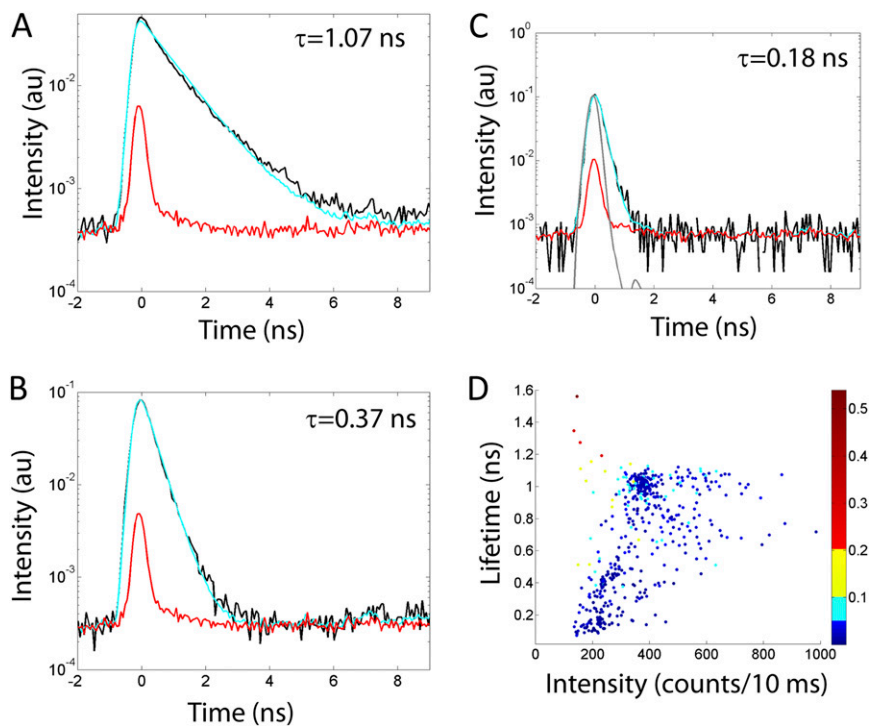


Fig. S4. Fluorescence lifetime fitting and calculation of confidence intervals. Representative fits of the fluorescence lifetime decay curves from single LH2 complexes are shown for state A (A), state B (B), and state C (C). The raw data are shown in black, with the best fit curve shown in cyan. The red curve shows the component due to background, and the gray curve in B shows the IRF. (D) Scatter plot showing a randomly selected subset of data from B800 excitation at $55.6 \mu\text{J}/\text{cm}^2$ per pulse, where the 95% confidence intervals have been calculated by bootstrapping the data 200 times for each point. The larger of the two differences from the calculated value is chosen, and shown for each point by the color scale.