

Article

Conformational Switching in a Light-Harvesting Protein as Followed by Single-Molecule Spectroscopy

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ABSTRACT Among the ultimate goals of protein physics, the complete, experimental description of the energy paths leading to protein conformational changes remains a challenge. Single protein fluorescence spectroscopy constitutes an approach of choice for addressing protein dynamics, and, among naturally fluorescing proteins, light-harvesting (LH) proteins from purple bacteria constitute an ideal object for such a study. LHs bind bacteriochlorophyll *a* molecules, which confer on them a high intrinsic fluorescence yield. Moreover, the electronic properties of these pigment-proteins result from the strong excitonic coupling between their bound bacteriochlorophyll *a* molecules in combination with the large energetic disorder due to slow fluctuations in their structure. As a result, the position and probability of their fluorescence transition delicately depends on the precise realization of the disorder of the set of bound pigments, which is governed by the LH protein dynamics. Analysis of these parameters using time-resolved single-molecule fluorescence spectroscopy thus yields direct access to the protein dynamics. Applying this technique to the LH2 protein from *Rhodovulum (Rdv.) sulfidophilum*, the structure—and consequently the fluorescence properties—of which depends on pH, allowed us to follow a single protein, pH-induced, reversible, conformational transition. Hence, for the first time, to our knowledge, a protein transition can be visualized through changes in the electronic structure of the intrinsic cofactors, at a level of a single LH protein, which opens a new, to our knowledge, route for understanding the changes in energy landscape that underlie protein function and adaptation to the needs of living organisms.

INTRODUCTION

Light-harvesting (LH) proteins gather solar photons during the first steps of the photosynthetic process and transfer the resulting excitation energy to the photosystems or reaction centers, where it is ultimately transformed into chemical energy (1). In purple bacteria the peripheral LH, or LH2, displays a variety of structures, however all are based on annular structures composed of pairs of membrane-spanning apoproteins, termed α and β . Each $\alpha\beta$ -dimer noncovalently binds three bacteriochlorophylls (Bchl) and one carotenoid molecule (2). The Bchl molecules are located in the hydrophobic space between the apoproteins, and form a weakly coupled ring (Fig. 1 A, left) responsible for the absorption at 800 nm (B800) of LH2, and a ring of strongly coupled molecules (Fig. 1 A, right) responsible for the absorption at 850 nm (B850) (Fig. 1 B).

Excitation energy resulting from the photon absorption by the carotenoid molecules present in LH2, or by Bchls from the B800 ring, is rapidly transferred to the B850 ring from

which it is either emitted (3–5) or, in the intact system, within a few ps transferred to an adjacent ring (LH1 or LH2) and finally to the reaction center (6,7). The electronic properties of the B850 ring are governed by the electronic coupling between its constitutive pigments. The magnitude of the nearest neighbor dipole-dipole coupling matrix elements were estimated to be between 300 and 400 cm^{-1} ((8,9) and references therein). Directly predicting the exciton manifold of this ring from the LH2 structure derived from x-ray crystallography leads to the unavoidable conclusion that the lowest excitonic transition would be forbidden, although the next two would be degenerate and carry almost all the oscillator strength (10). In reality, LH2 provides an anisotropic and flexible environment to the B850 BChl molecules, the dynamics of which proceeds on numerous timescales. Pigment-protein and pigment-pigment interactions are accordingly time-dependent, and thus the Bchl site energies and Bchl-Bchl excitonic couplings fluctuate with time. This results in an inhomogeneous broadening of the LH2 electronic transitions, and, due to the competition between energetic disorder and excitonic coupling, both being of similar magnitude (11), the lowest transition of the LH2 ring is not forbidden, it is allowed and actually superradiant. The primary biological function of LH2 proteins—efficient energy transfer toward reaction

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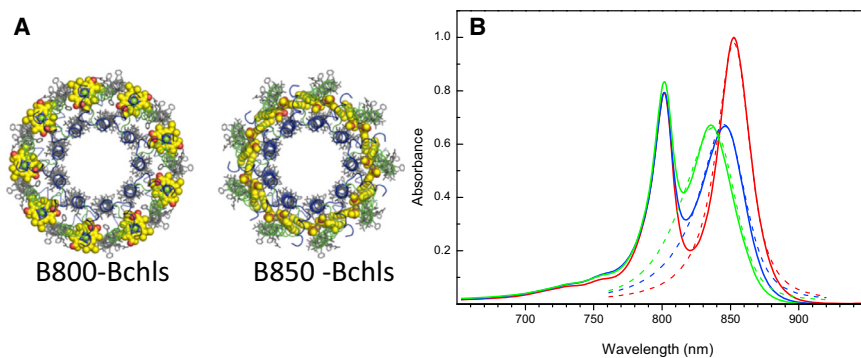


FIGURE 1 The structure and steady-state absorption properties of LH2. (A) Cartoon structure of LH2 looking in a direction parallel to the direction of the transmembrane α -helices from the cytoplasmic side. The bacteriochlorophyll rings, B800 (left) and B850 (right), are depicted in space-fill mode and are sandwiched between the nonameric ring of α - (blue backbone) and β - (green backbone) polypeptides. Derived from RCSB Protein Data Bank file 1NKZ. (B) 287 K bulk absorption spectra of *Rhodovulum sulfidophilum* strain DSM 1374 LH2 at pH 8.5 (red trace), pH 7.5 (blue trace), and pH 7.0 (green trace). The broken lines represent the calculated B850 absorption spectra using the Alternative Redfield theory.

centers—thus essentially relies on the disorder in their structure.

Although low-temperature single-molecule studies have characterized the LH2 system (e.g. (12–15)) the dynamic disorder of LH2 has largely been documented by studying the fluorescence dynamics of individual LH2 complexes at ambient temperature under continuous illumination in the Q_x -Bchl electronic transition (16–19), and to a lesser extent using pulsed illumination (17,20,21). At ambient temperature, single complexes generally display reasonably steady fluorescence properties, however with occasional reversible spectral jumping either to the blue or to the red, accompanied by changes in spectral shape, as well as intermittency (i.e., blinking behavior). It was further shown that this behavior, which obviously arises from complex protein structural diffusive motions, may be modeled assuming that each pigment in the tightly coupled ring displays two (22) or better four states of electronic transition energy (23).

One specific property of the LH2 proteins from *Rhodovulum (Rdv.) sulfidophilum* (formally *Rhodobacter sulphidophilus*) is that they undergo a blueshift of their lower electronic transition due to a pH-induced breakage of the H-bonds between the acetyl carbonyl of the B850-absorbing Bchl and their neighboring Trp₄₄ and Tyr₄₅ amino acids from the α polypeptide while still maintaining the overall quaternary structure (24–26). This phenomenon, specific to these complexes, has been attributed to the presence of glutamic acid residues, which are present only in the *Rdv. sulfidophilum* sequence, and which are assumed to become protonated at low pH. The pH-induced spectral changes of these complexes are gradual, and when fully achieved at pH 7.0, the absorption transition of the B850 ring peaks at 832 nm, thus undergoing an 18 nm blueshift. At pH 7.5, the lower energy electronic absorption transition of the complexes is already broadened and blueshifted, peaking at 841 nm (Fig. 1 B). Hence, in this work we applied single-molecule fluorescence spectroscopy to the LH2 complexes from *Rdv. sulfidophilum* to observe the pH-induced conformation transitions—including the reversible titration by pH of a single protein—in relation to the dynamic disorder of this biological system.

MATERIALS AND METHODS

Cell culturing and protein isolation

Liquid cultures of *Rdv. sulfidophilum* strain DSM 1374 were grown at pH 6.8 in modified Rhodospirillaceae medium No. 27 (<http://www.dsmz.de>) at $28 \pm 2^\circ\text{C}$ in glass bottles located between banks of incandescent lamps. Photosynthetic membranes were prepared and the LH2 proteins purified in the presence of the detergent *N,N*-Dimethyldodecylamine *N*-oxide (LDAO, Fluka, Buchs, Switzerland) essentially as previously described (32,33). The LH2 proteins were stored in 100 mM NaCl, 0.05% (w/v) LDAO, 20 mM Tris.HCl, pH 8.5. Blueshifted spectral forms of LH2 were obtained by modifying the pH and anionic strength as previously described (24–26) (either 15 mM, pH 7.5, or 25 mM CsCl, pH7.0), by dialysis against a second buffer while maintaining the same detergent and Tris concentrations, or by slowly flushing the sample chamber of the fluorescence spectrometer with a second deoxygenated buffer (see below) and allowing time for the protein sample to equilibrate. In the second approach careful attention was made to ensure that the final spectral properties were comparable to those of the bulk dialysis approach. The protein samples were immobilized on a Poly-L-lysine (Sigma-Aldrich, Schnelldorf, Germany) coated glass coverslip situated inside a homemade, temperature-controlled sample cell as previously described (17). The protocol for obtaining a monolayer of immobilized proteins in a deoxygenated buffer—using the cocktail of catalase (35 mg/ml, Sigma-Aldrich), glucose oxidase (200 mg/ml, Sigma-Aldrich), β -D-glucose (7.5 mg/ml, Sigma-Aldrich), and dry nitrogen flux—has been described in detail elsewhere (18,34).

Spectroscopy

278 K absorption spectra were recorded using a Cary 5E spectrophotometer (Varian plc, Sydney, Australia). Fluorescence images and spectra were acquired with an inverted confocal microscope (Nikon, Eclipse TE300 (Amsterdam, The Netherlands)), as previously described (17,34). The excitation source was a constant power He-Ne laser (Melles Griot, 05SYR810-230 (Voisins le Bretonneaux, France)) with randomly polarized emission. The excitation wavelength of 594 nm (at 2 μW) permitted direct excitation of the Q_x transition of all the Bchl molecules in LH2. A dichroic beam splitter (Chroma Technology, 605dxt (Rockingham, VT)) reflected the laser beam into the objective lens (Nikon, Plan Fluor 100 \times , 1.3 NA, oil immersion), focusing the excitation light onto the glass-water interface in the sample cell to a full width at half-maximum (FWHM) of ~ 600 nm. The intensities used in these experiments represent the values at this interface. The fluorescence emission was filtered with a 100 μm pinhole and a long-pass glass filter RG715 (Edmund Optics (York, UK), 46065). The sample cell was mounted on a closed loop two-dimensional piezo stage (Physik Instrumente, P-731.8C) controlled by a digital four-channel controller (Physik Instrumente, E-710.4LC (Karlsruhe,

Germany)), and the temperature maintained at 287 K by a regulated circulating water bath. To obtain images, emission was detected with a Si avalanche photodiode single photon-counting module (SPCMAQR- 16, Perkin-Elmer (Waltham, MA)), controlled by a counter timer board (National Instruments, PCI-6602 (Woerden, The Netherlands)). Spectra were acquired by dispersing the fluorescence onto a liquid nitrogen-cooled back-illuminated charge-coupled device (Princeton Instruments, Roper Scientific, Spec10: 100BR (Vianen, The Netherlands)); pixel binning yielded a resolution of <0.8 nm.

Images and spectra

A FL image was acquired by continuously sweeping the piezo stage over the laser focus with a frequency of 3 Hz while its position in the perpendicular direction was changed by 100 nm for each line; the FL signal was concomitantly detected with an avalanche photodiode (Perkin Elmer, SPCM-AQRH). Images were then constructed by associating the piezo stage coordinate with the corresponding intensity. The scanning covered a $10\ \mu\text{m} \times 10\ \mu\text{m}$ area. After the coordinates of bright particles were determined, the piezo stage was positioned to bring the particle into the focus of the objective, and after the mode was switched to a spectroscopic one, a series of FL spectra were collected for 30 s, or longer, with an integration time of 1 s per spectrum.

Data analysis

Each measured FL emission spectrum in the time series was fitted with a skewed Gaussian function as previously described by applying a least squares fitting procedure (17,34,35). The expression for the skewed Gaussian function is

$$F(\lambda) = \Delta + A \exp\{-\ln(2)/b2\ln[1 + 2b(\lambda-\lambda_m)/\Delta\lambda]\}^2,$$

where Δ is the offset, A the amplitude, λ_m the fluorescence peak (FLP) wavelength, $\Delta\lambda$ the width, and b the skewness. The FWHM of the spectrum was calculated from the width and the skewness. Consequently, by fitting each spectrum from a series, we obtained the time traces of the amplitude, the FWHM, and the FLP with the corresponding confidence margins. In some cases two skewed Gaussian functions were required to fit a spectrum because individual proteins often exhibited faster dynamics than that of the integration time (36). The fluorescence spectra were quantitatively reproduced using the Redfield exciton model that was previously developed for other LH2 complexes (4,18,23,31).

RESULTS AND DISCUSSION

Fig. 1 B displays the electronic absorption spectra of LH2 complexes purified from *Rdv. sulfidophilum* at pH 8.5, 7.5, and 7.0. As previously published (24) lowering the pH from 8.5 to 7.0 induces ~20 nm blueshift of the lower energy transition of these complexes from 853 to 833 nm, although the carotenoid-absorbing region is unperturbed indicating that the overall quaternary structure is conserved (see Fig. S1 in the Supporting Material). In the pH range 8.0 to 8.5, comparable results are obtained when performing similar single-molecule fluorescence experiments at room temperature with LH2 complexes purified from *Rdv. sulfidophilum* (Fig. 2) to those previously isolated from *Rhodoblastus (Rbl.) acidophilus* (formally *Rhodopseudomonas acidophila*) (4,17) and *Rhodobacter sphaeroides* (18,19). In these three species the quaternary structure

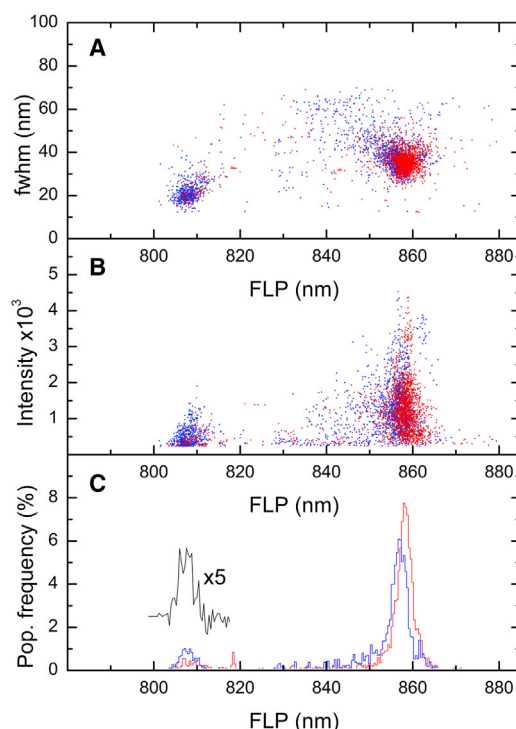


FIGURE 2 The distribution of FLP position as a function of (A) FWHM, (B) Intensity (counts per second), and (C) relative population of fluorescent emitting states in *Rdv. sulfidophilum* LH2 proteins at pH 8.5 (red) and pH 7.5 (blue); the pH 8.5 data set is superimposed over the pH 7.5 results. (C) Inset: difference spectrum of LH2_{pH 7.5} minus LH2_{pH 8.5} ($\times 5$) highlighting the increase in blueshifted FLP positions at pH 7.5. The LH2 proteins were poised at pH 8.5 in the sample chamber and the pH reduced to 7.5. After equilibration, a series of spectra (30×1 s) were measured for 131 LH2 proteins. The same sample was then titrated with the original pH 8.5 buffer to reinstate the original LH2 spectral form and the fluorescence measurements repeated for a similar number (139) of proteins. $T = 287$ K.

(2,27,28) and overall Bchl H-bond network (3,26,29,30) of the LH2 proteins are very similar. The peak position of the observed fluorescence spectrum from a single *Rdv. sulfidophilum* LH2 appears mainly at 858 nm, with a bandwidth of 32 nm (i.e., similar to that observed for the bulk (24)).

Once equilibrated to a pH of 7.5 the spectral dynamics of 131 LH2 proteins were measured; this was followed by buffer titration to pH 8.5 and a second series of spectra acquired. At the very low intensity used to induce the fluorescence, only limited spectral jumps are observed (e.g., Fig. 2 A, red dots), most of them within ± 5 nm. A low but statistically significant fraction of jumps leading to fluorescence emission centered at around 808 nm is observed. It must be noted that, although some of the complexes may eventually jump back from this position to the normal 858 nm fluorescence, the transition to such a blue position of the fluorescence emission is often accompanied by a complete, irreversible quenching of the complex during the timescale of the experiment.

Knowing that under our experimental conditions at pH 8.5 limited spectral jumping occurs in the LH2 from *Rdv. sulfidophilum* the sample chamber was loaded with protein at this pH. The pH was reduced in situ to 7.5 and fluorescence spectra and 131 individual LH2 proteins measured. A summary of the data analysis is presented in Fig. 2. This pH was chosen as it is expected to bring the protein close to the conformational change saddle point, and thus observing single LH2 in these conditions should yield the richest dynamic information. On the other hand, in our measurement conditions, the protein is clearly much less stable at pH 7.5, which makes the analysis of the results difficult. In these conditions, the center of gravity of the fluorescing population is blueshifted by 3 nm (Fig. 2, blue dots) when compared to the LH2 population at pH 8.5. The distribution of fluorescence emission maxima is broad (Fig. 2 A), and asymmetric, extending down to 830 nm with decreasing average intensities (Fig. 2 B). Between 854 and 830 nm, the width of the observed fluorescence transition observed increases (on average, see Fig. 2 A) according to their spectral position, indicating that the complexes explore a larger conformational space during the time of our measurements when fluorescing at shorter wavelengths. The number of spectra exhibiting FLP positions at ~811 nm is significantly greater at pH 7.5 than at pH 8.5 (Fig. 2 C, as emphasized by the difference spectrum) as well as their average intensity (Fig. 2 B), reflecting an increased dwell time at this position relative to the temporal window of the experimental apparatus (1 s) at this pH. When reinstating pH 8.5 in the buffer chamber, and without changing the protein sample, the 850 nm absorption properties are restored and the results from single-molecule experiments from another 139 proteins (Fig. 2, red dots) are similar to those measured before changing the pH to 7.5, showing that it is possible to reversibly titrate LH2 from *Rdv. sulfidophilum* in the single-molecule fluorescence spectrometer under physiological relevant conditions without inducing intrinsic artifacts.

It is actually possible to follow, during this experiment, how each protein responds to the pH changes and explores a larger conformational space. Fig. 3 displays trajectories at pH 7.5 for single complexes upon lowering the pH. In Fig. 3, the width (which is a combination of homogeneous and inhomogeneous broadening) actually reflects the number, but also the relative time, of differently fluorescing sites the protein is exploring during the time of measurement, whereas the peak position yields information only when the width of the transition becomes narrower. A large variety of spectral dynamics can be observed. As demonstrated in Fig. 3, A–D, lowering the pH before data acquisition induces a dramatic increase of the linewidth of the fluorescence transition (as mentioned previously for Fig. 2), indicating that, over the characteristic timescale of our experiment (30 s), the protein wanders from the saddle point into its enlarged conformational space. Occasionally extremely large fluorescence bandwidths are observed in Fig. 3 which, in general,

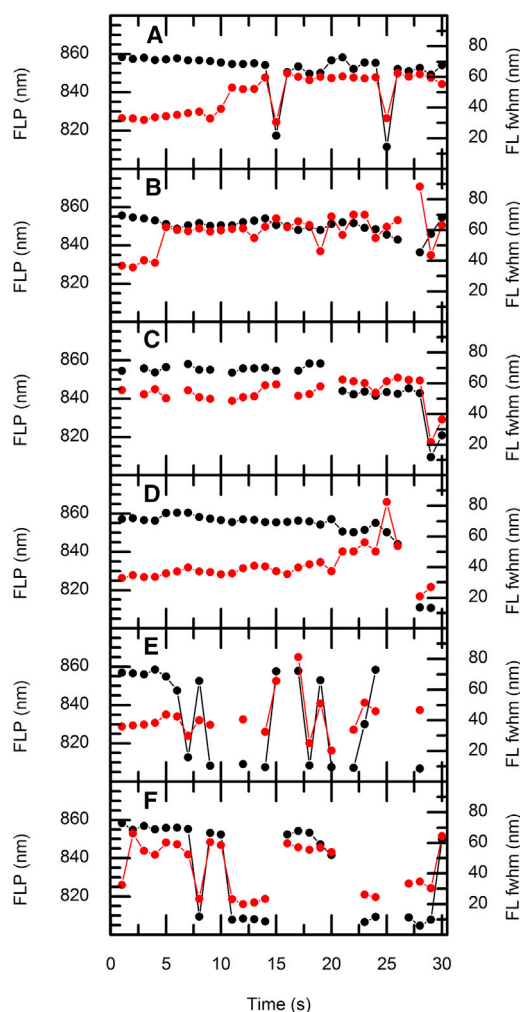


FIGURE 3 Temporal traces of the maximum FLP position (*black traces*) and width (FWHM, *red traces*) of six LH2 proteins (A–F) equilibrated in the pH 7.5 buffer. The Gaps in the traces are due to fluorescence intermittency (i.e., quenched states when no fluorescence emission is observed). $T = 287$ K.

correspond to spectra that contain two peaks (due to the temporal resolution of the experimental apparatus). A fast shift to higher energy of this transition, accompanied by a reduction of its width (Fig. 3 A), indicates the presence of a state fluorescent at ~820 nm, which does not correspond to protein unfolding as the fluorescence is rapidly restored to the near-to-native position. High-energy emission is often associated with an emission band at ~811 nm, sometimes in equilibrium with nonemitting states (see Fig. 3, E and F), which may last for seconds before the protein returns to a close-to-native emitting state.

In a number of cases, we observe a change of a single protein very reminiscent to what is observed in the bulk, which may be reversibly titrated from pH 8.5 to pH 7.0 and back. An example of such a titration is shown in Fig. 4 where we followed the fluorescence emission for 90 s, which summarizes Movie S1 in the Supporting Material. For clarity, the

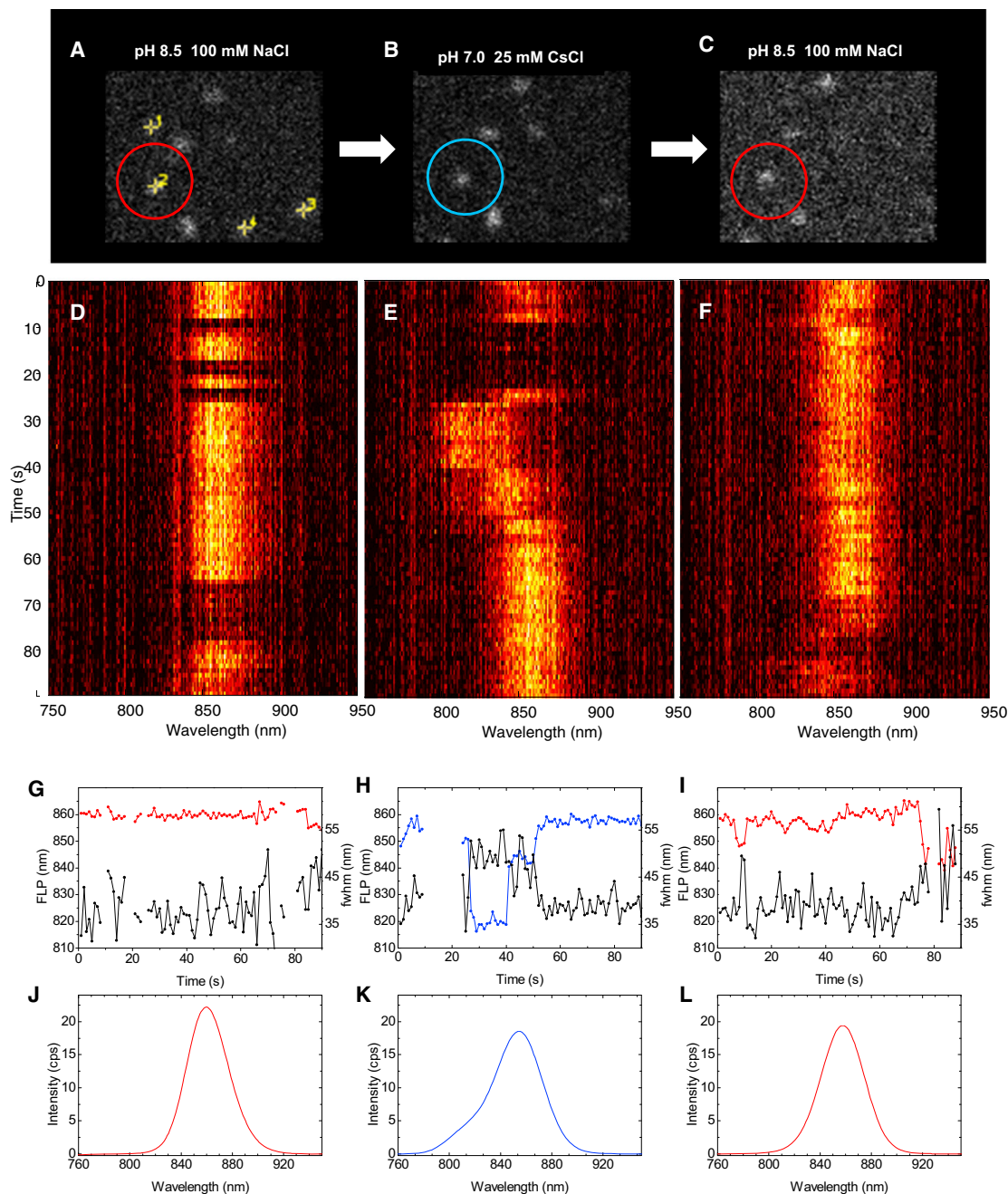


FIGURE 4 Reversible titration of a single LH2 complex. Under each condition the LH2 protein was allowed to equilibrate, and the fluorescence emission spectra collected for 90 s. The panels should be read vertically from left to right: LH2_{pH8.5} (A, D, G, and J), LH2_{pH7.0} (B, E, H, and K), and return to the original LH2_{pH8.5} state (C, F, I, and L). The circled molecule in the raster scans (A–C) was used for the fluorescence measurements. (D–F) 2D plots of FLP position and intensity as a function of illumination time. (G–I) Calculated FLP (left axis, black traces) and peak FWHM (right axis, colored traces) as a function of illumination time. The Gaps in these traces are due to spectral intermittency, or blinking behavior (i.e., quenched states when no fluorescence emission is observed). (J–L) Average fluorescence spectrum. T = 287 K.

fluorescence spectra in [Movie S1](#) have been vertically stacked.

At pH 8.5, although the protein shows fluorescence intermittency, it undergoes no apparent major spectral jump and emits at 860 nm. When the same LH2 is titrated to pH 7.0 there is a dramatic increase in the observed spectral

dynamics, reflecting major spectral fluctuations of the emitting chromophores, with some well-defined states emitting at 845 nm, and some emitting at 820 nm. The fluorescence behavior of the very same protein returns to its original state when the pH 8.5 is restored, (Fig. 4, F, I, and L), indicating that we could induce it to reversibly explore a very large

conformational space. It should be noted that when pH 8.5 is restored a minor blueshift observed at ~ 80 s is actually due to a low-intensity, near fully quenched state (see [Movie S1](#)). We can thus safely analyze the protein dynamics at pH 7.0 as reflecting a genuine, pH-induced, conformational change, for the first time observed at the level of a single protein without the aid of externally tagged fluorophore markers. In this particular case, it is surprising that the spectral evolution observed at pH 7.0 involves for most of the time fluorescence transitions only 15 nm broader ([Fig. 4 H](#)) than the native emission spectrum, indicating that it apparently implicates a stepwise pathway with defined intermediate steps lasting for seconds. The full conversion requires times longer than 1 s to be completed. On the way back from the substate emitting at 820 nm, the 855 nm FLP is restored through a second, stable, substate emitting at 840 nm, which lasts for ~ 10 s. Again, the transition time between the 840 and 855 nm emission states occurs over a number of seconds.

Our results allow, for the first time, to our knowledge, the visualization of the dynamic behavior at the saddle point of a single protein in the transition state, resulting in the exploration of the conformational space of both conformations, within a timescale shorter and on the order of that of our experiments. Furthermore, in a limited number of cases, we are able to identify substates on the conformational transition path, which under our experimental conditions are stable for seconds. Empirically from the data, there are two stable emitting states in *Rdv. sulfidophilum* LH2. The first state is the 860 nm emitting state and is where all the Bchls in the ring are considered to be in a near similar low-energy state, but still sensitive to fluctuations in the local disorder. Thus, this explains the tight cluster of fluorescence data points in [Fig. 2 A](#) where the emission wavelengths are centered at ~ 860 nm an average FLP FWHM of ~ 32 nm. The second stable state occurs when all the Bchl pigments exhibit an even tighter cluster of data points, with emission wavelengths centered at 811 nm, and is associated with a significantly narrower FLP FWHM of ~ 25 nm. In this more stable state all the Bchl molecules in the LH2 ring are considered to be in a quasisimilar high-energy state. There are relatively few substates in proximity to this second cluster. However, there are numerous intermediate states where the fluorescence emission wavelengths are located between 860 and 840 nm (hereon called the 840 nm substate), with a general broadening of the fluorescence bandwidth as one moves to lower wavelengths. Of note, there is no evidence to support a single intermediate state, but rather a continuous distribution of substates (see [Fig. 2 C](#)). This implies that as the FLP maximum blueshifts from 860 nm toward the 811 nm state, via the 840 nm substate, that there is an increasing number of high-energy state Bchls within the ring.

The B850 absorption spectra of *Rdv. sulfidophilum* LH2 at pH = 8.5 can be reproduced using the same exciton model

that has been used for quantitative fits of other LH2 complexes ([4,18,23,31](#)). Most of them are characterized by a large spectral disorder. Initially, a spectral disorder of 500 cm^{-1} was used; however, an alternative solution was found where this value was reduced to 250 cm^{-1} , with a corresponding increase in phonon coupling. Although both models gave almost undistinguishable fits of the bulk absorption spectra the statistics of spectral fluctuations observed in the subsequent SMS experiments (see [Supporting Material](#) and [Fig. 2 A](#)) can be reproduced only by the alternative model, with its smaller disorder of 250 cm^{-1} , and it is these calculated absorption spectra that are plotted in [Fig. 1 B](#). Here, only relatively small spectral shifts of individual pigments were required to fit the spectra, and similar to those previously obtained for *Rbl. acidophilus* ([18](#)). This was confirmed by optimally fitting the absorption spectra at pH 7.0 and 7.5, where a blueshift of the Bchl-850 of some 500 cm^{-1} was given the probability of 65% and 45%, respectively, over and above the small jumps (mostly within 10 nm) present in the pH 8.5 sample. Indeed, we remind the reader that the minimal model capable to explain the SMS dynamics for *Rbl. acidophilus* LH2 includes one coordinate with two conformational states, shifting the site energies by 190 cm^{-1} to the blue or to the red, and second coordinate with two more conformational states, creating bigger shifts, i.e., 440 cm^{-1} to the blue or to the red (the so-called four-state model ([23](#))). Hence, The minimal model for *Rdv. sulfidophilum* should be a three-state one (see [Supporting Material](#)), consisting of two states responsible for small spectral fluctuations and the third one, which is pH-dependent, with larger ($\sim 500\text{ cm}^{-1}$) spectral blueshifts.

[Fig. 5](#) reflects our current understanding of the substates observed during the conformational changes observed in LH2 primarily as a function of ΔpH . It is clear that other single-molecule fluorescence measurements on the LH2

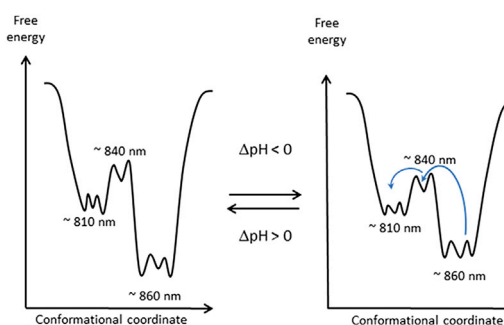


FIGURE 5 Schematic representation of the observed conformational changes in the protein energy landscape of LH2. (*Left*) Energy landscape associated with a high pH environment (8.5). Large barrier height between local minima associated with 810 and 860 nm emission points to infrequent transitions between these states. (*Right*) Lowering the pH and/or salt concentration may increase the free energy of the 860 nm local minimum, thereby increasing the transition frequency to conformational states associated with higher energy emission. The conformational substate associated with 840 nm emission is on the transition pathway to the more stable 810 nm state.

protein from *Rdv. sulfidophilum* that embrace a broader range of physiologically relevant parameters—temperature, pH, ionic strength, and the role of the hydrophobic medium—are pertinent. Such experiments will provide a detailed and statistical account of the protein transition pathway, via the stable intermediate substates, and will allow us to establish a better understanding of protein energy landscapes (see Fig. 5).

SUPPORTING MATERIAL

Supporting Materials and Methods, two figures, and one movie are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(15\)00396-3](http://www.biophysj.org/biophysj/supplemental/S0006-3495(15)00396-3).

AUTHOR CONTRIBUTIONS

A.G., B.R., and R.v.G. designed research; A.G., C.I., and T.P.J.K. performed research; A.G., C.I., T.P.J.K. and V.I.N. analyzed data; A.G., C.I., T.P.J.K., B.R., and R.v.G. wrote the article.

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Supporting Material.

Conformational Switching in a Light-Harvesting Protein as Followed by Single-Molecule Spectroscopy

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Absorption spectroscopy.

Fig. SI1 displays the UV-vis-NIR electronic absorption spectra of LH2 complexes purified from *Rdv. sulfidophilum* equilibrated in buffers at pH 8.5, 7.5 and 7.0. As previously published (1) lowering the pH from 8.5 to 7.0 induces a ~20 nm blue shift of the lower energy transition of these complexes from 853 to 833 nm, while the carotenoid-absorbing region is unperturbed indicating that the overall quaternary structure is conserved ².

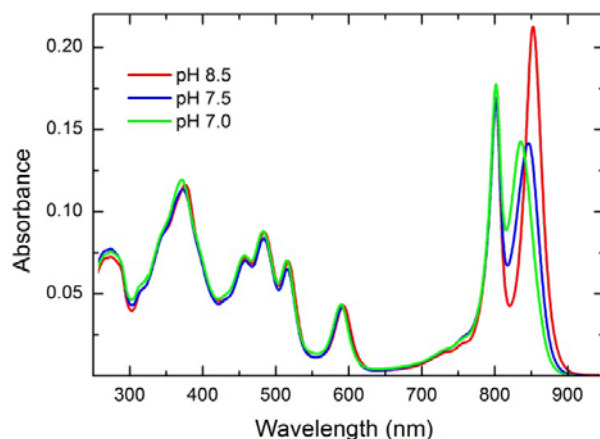


Fig. SI1. Room-temperature UV-vis-NIR electronic absorption spectra of LH2 complexes purified from *Rdv. sulfidophilum* equilibrated in buffers at pH 8.5, 7.5 and 7.0.

Redfield exciton model.

The NIR electronic spectrum of LH2 from *Rdv. sulfidophilum* at pH=8.5 can be reproduced using the same exciton model that has been employed to quantitatively fit other LH2 complexes (3-5). Most of the previous studies were characterized by a spectral disorder of the order of 500 cm^{-1} . Initially a value of 500 cm^{-1} was used for the disorder; however, this was reduced to 250 cm^{-1} in an alternate model. In this case, the reduced disorder was concomitant with an increase in phonon coupling. Both models gave similar fits of the bulk absorption spectra, but the statistics of spectral fluctuations observed in the SMS experiment can be reproduced only by the alternative model with its small disorder.

In Fig. SI2 the statistics of the fluorescence band width (fwhm) are plotted as a function of the peak position for the two exciton models as well as the experimental data at pH=8.5 (red) and pH=7.5 (blue). In the first example we compare the measured statistics with the normal model with a large disorder of 500 cm^{-1} (3). Such a model describes well the SMS

dynamics observed for LH2 complexes from different bacteria (see Ref. 3). Besides jumps with small amplitudes (within 10 nm) there are big jumps to the blue and to the red (the latter case is characterized by big broadening and changes in asymmetry of the fluorescence band). Small and big jumps correspond to a two conformational coordinates producing the shifts of the site energies that are smaller than, or comparable to, the pigment-pigment coupling ($250\text{--}300\text{ cm}^{-1}$ for the B850 band), or significantly exceed it (thus producing dramatic changes in the exciton structure of the complex) (6). However, in the case of *Rdv. sulfidophilum*, with its additional pH-dependent blue-shift, such a model doesn't work as the distribution of fluorescence band width is too large.

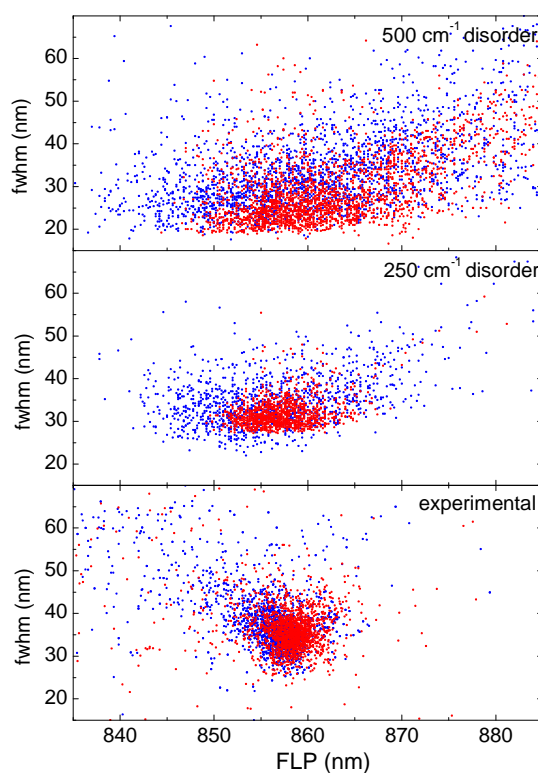


Fig. SI2. Comparison of the width (fwhm) of the fluorescence peak (FLP) position obtained from the two exciton models having disorder values of 500 cm^{-1} (top) and 250 cm^{-1} (middle) with the experimental data (bottom) at pH = 8.5 (red) and pH = 7.5 (blue).

Looking at the experimental data we may conclude that only small jumps (mostly within 10 nm) are present in *Rdv. sulfidophilum* LH2 when poised at pH=8.5. So, probably only one conformational coordinate is active, responsible for relatively small spectral shifts of individual pigments (i.e. no more than 300 cm^{-1}). This situation can be modelled by imposing

the disorder value of 250 cm^{-1} in our alternative model. We can further suppose that lowering in pH activates another conformational coordinate that can produce big spectral shifts, more specifically, the 500 cm^{-1} blue-shifts. Such a model is not only consistent with the pH-dependent bulk spectra (see Fig. 1), but also gives better agreement with the measured statistics.

The minimal model capable to explain the observed SMS dynamics for *Rbl. acidophilus* LH2 includes one coordinate with two conformational states, shifting the site energies by 190 cm^{-1} to the blue or to the red, and second coordinate with two more conformational states, creating larger shifts, *i.e.* 440 cm^{-1} to the blue or to the red (the so-called four-state model (6)). The minimal model for *Rdv. sulfidophilum* LH2 should be thus a three-state one, including the two states responsible for small spectral fluctuations and the third state (pH-dependent) with the big (about 500 cm^{-1}) blue shift. In this work a more general description of small shifts (Gaussian distribution instead of just two discrete states) was employed, but still restricted to a just one discrete conformational state responsible for large blue shifts.

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