Supporting Information

How Deep is the Potential Well Confining a Protein in a Specific Conformation? A Single-Molecule Study on Temperature Dependence of Conformational Change between 5 and 18 K

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S1. Determination of the rate of the change of the absorption frequency of a single chromophore

The rate of the spectral change is determined by counting the number of discrete jump of the absorption frequency in a unit time. Since the absorption frequency is determined by curve fitting of a spectrum, the fitting error of the frequency causes artificial frequency jumps. The dark noise of the detector and the background that are independent of laser frequency do not cause an error in the center frequency of the absorption, because the experimental data is fit to a fixed lineshape of Lorentzian. The inevitable fitting error arises from the finite number of photons that are detected in a single scan across the absorption peak. Statistical distribution of the photons under the absorption lineshape results in an error of the center frequency of the fitting. The amount of the error depends on the total number of photons and the linewidth. In the journal the error of 5 cm^{-1} is used as the threshold of detectable jumps. The procedure to obtain this value of 5 cm^{-1} is given in the following.

S1.1. Fitting error due to photon statistics

Let us estimate the fitting error by evaluating the fluctuation of the frequency that separates the total signal photons into equal halves. Let us assume that a Lorentzian peak of absorption consisting of N photons is centered at frequency ν of zero (see Figure S1A). When the number of photons in the left and right areas are denoted by A_1 and A_2, respectively, A_1 and A_2 are statistically independent quantity having the same average (avg) μ of $N/2$ and the same standard deviation (sdev) of σ. The mean square of the deviation of the half intensity $A_1/(A_1 + A_2)$ from 1/2 is

$$\Sigma^2 = \left\langle \left(\frac{A_1}{A_1 + A_2} - \frac{1}{2} \right)^2 \right\rangle = \frac{1}{4} \times \frac{\left\langle A_1^{\,2} \right\rangle - \left\langle A_1 \right\rangle^2}{\left\langle A_1^{\,2} \right\rangle + \left\langle A_1 \right\rangle^2} = \frac{1}{4} \times \frac{\sigma^2}{2\mu^2 + \sigma^2} \qquad \text{(S1)}$$

where the angle brackets indicate statistical average. Since emission of photons follows Poisson statistics in which $\sigma = \sqrt{\mu}$, Σ^2 is rewritten as

$$\Sigma^2 = \frac{1}{4} \times \frac{1}{2\mu + 1} \cong \frac{1}{8\mu} \qquad \text{if} \qquad \mu >> 1 \tag{S2}$$

Thus the sdev of the half intensity Σ is

$$\Sigma = \frac{1}{2\sqrt{N}} \tag{S3}$$

Translation of sdev of the half intensity Σ into sdev in frequency $\delta\nu$ is accomplished by making a rectangle that compensates the deviation of intensity from 1/2 (see Figure S1B).

$$\Sigma = f(0)\delta\nu \tag{S4}$$

where $f(0)$ is the value of the normalized lineshape function $f(\nu)$ at the center frequency of $\nu = 0$. Since $f(0) = 2/(\pi\Delta\nu)$ in the normalized Lorentzian with the linewidth $\Delta\nu$ in the full width at half maximum, the expected error of the center frequency $\delta\nu$ is

$$\delta\nu = \frac{\pi}{4\sqrt{N}}\Delta\nu \tag{S5}$$

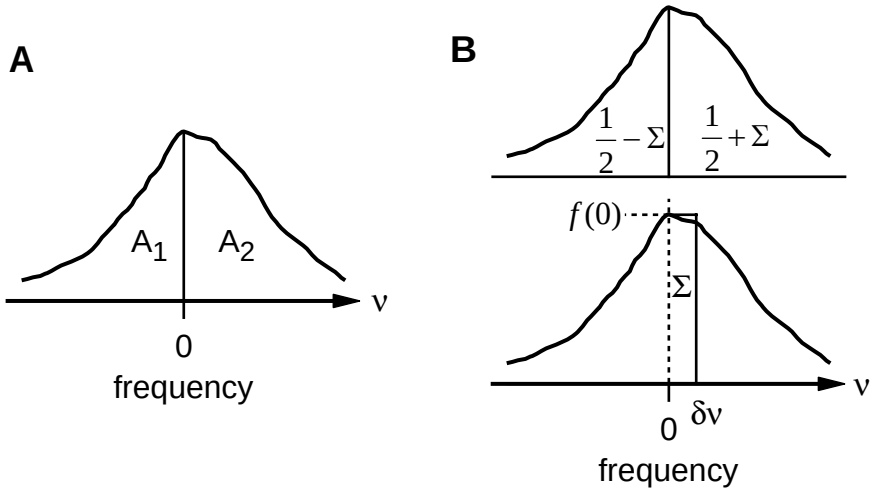

Figure S1.

S1.2. Instantaneous linewidth of the absorption of single chromophores

Since the fitting error $\delta\nu$ is proportional to the linewidth (see eq.(S5)), the value of $\Delta\nu$ is necessary. The linewidth $\Delta\nu$ corresponds to the instantaneous width during a single scan across the peak. The width can be obtained from the average spectrum of all the scans in which each spectrum is shifted by the amount of the fitted center frequency so that the effect of the temporal change of the center frequency is removed. Figure S2 shows examples of such an averaged spectrum. The linewidth deduced from all the colored trajectories is plotted in Figure S3. The same color as in the journal is used to refer to individual chromophores. The trajectory of the blue shows little temperature dependence (Figures 2 and 3). Correspondingly, the linewidth of the averaged spectrum at 18 K is about the same as the linewidth at 4.7 K The rate of the spectral jumps in the trajectory of the yellow increases with temperature (Figures 2 and 3). In Figure S2 the linewidth of the averaged spectrum also increases with temperature. In the averaged spectrum at 18 K contributions from nearby transitions appear as extra peaks.

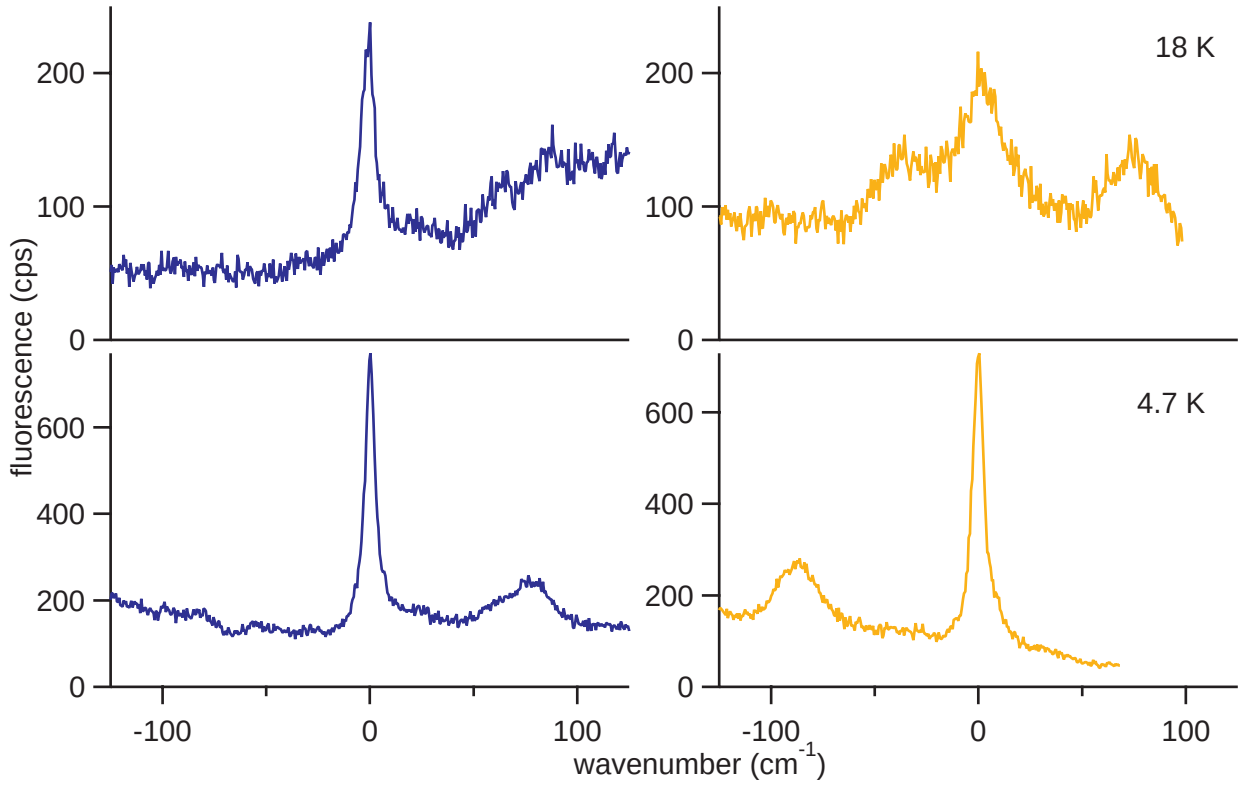

Figure S2.

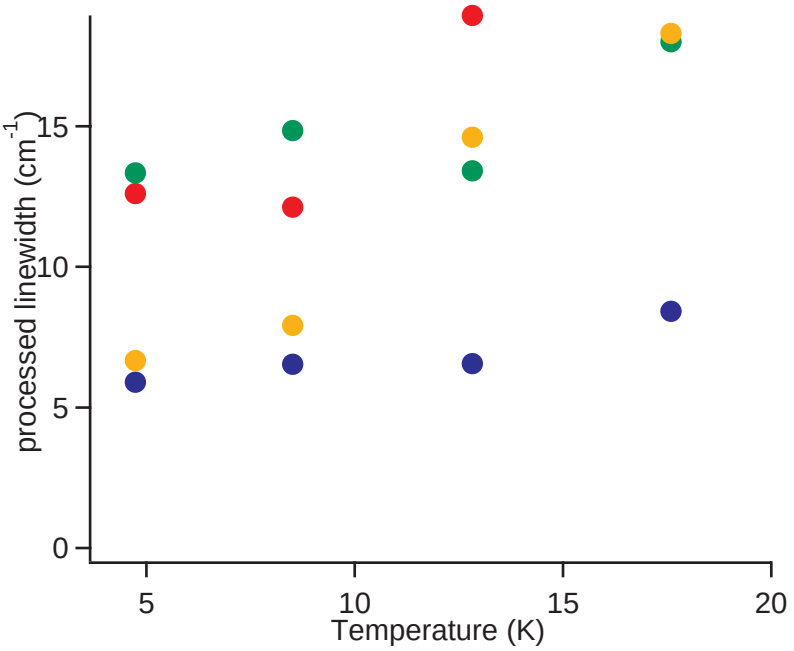

Figure S3.

S1.3. The minimum detectable frequency jump

Now that the instantaneous linewidth is known for all the analyzed trajectories, the threshold can be set to determine the minimum detectable frequency jump. The worst accuracy of the fitted frequency comes from the broadest and weakest absorption, that is the yellow trajectory at 18 K. With $N = 10$ and $\Delta\nu = 20$ cm^{-1}, we obtain 5 cm^{-1} as the threshold.

S2. The additional experimental data

Figure S4 show the fluorescence excitation spectrum of two individual complexes at several different temperatures. Trajectory of the fitted absorption frequency is indicated by the colored lines. These data are not shown in the journal but included in the histogram of Figure 3B.

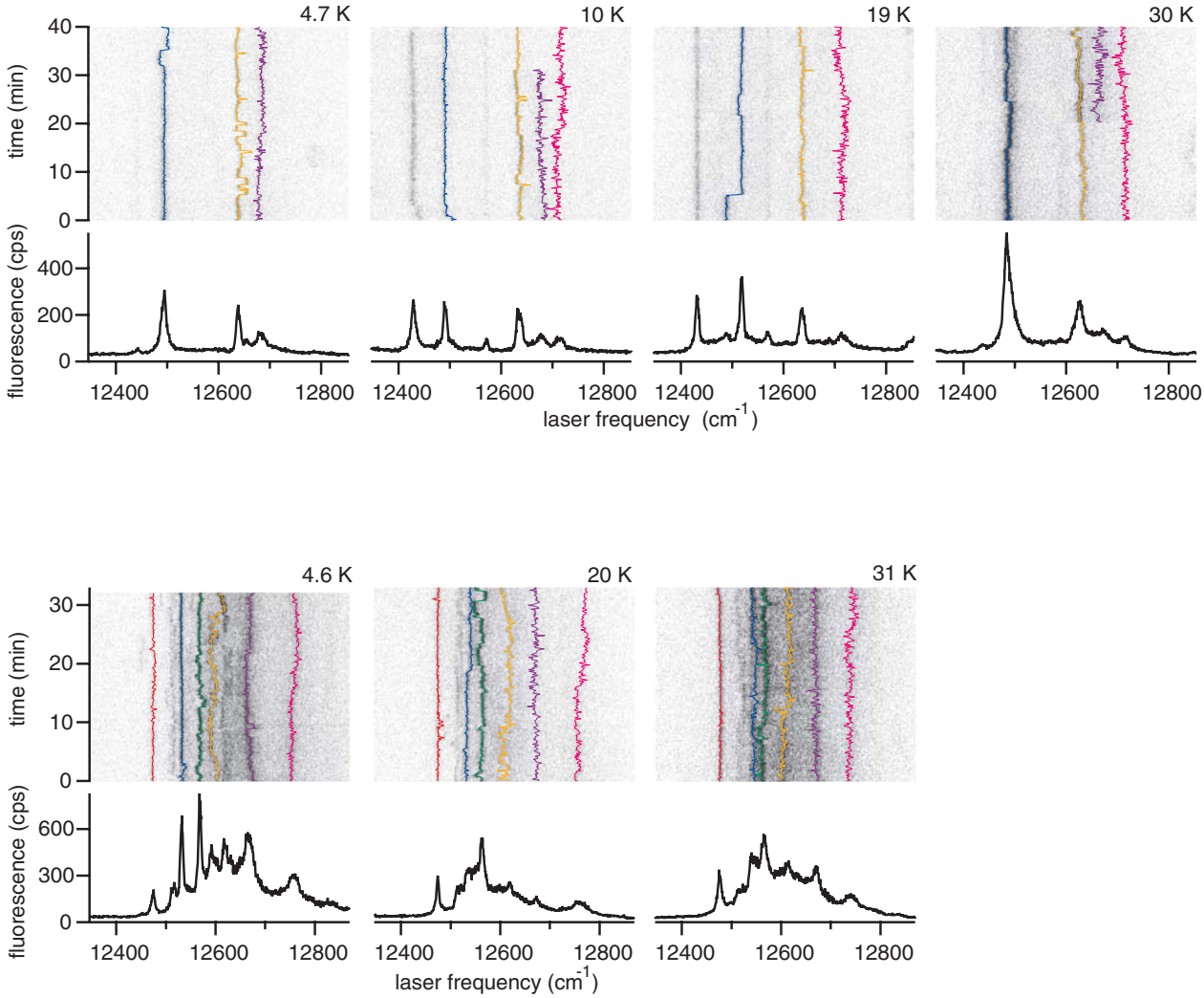

Figure S4.