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Supporting Information

Analysis of Fluorescence Lifetime and Energy Transfer Efficiency in Single-Molecule Photon Trajectories of Fast-folding Proteins

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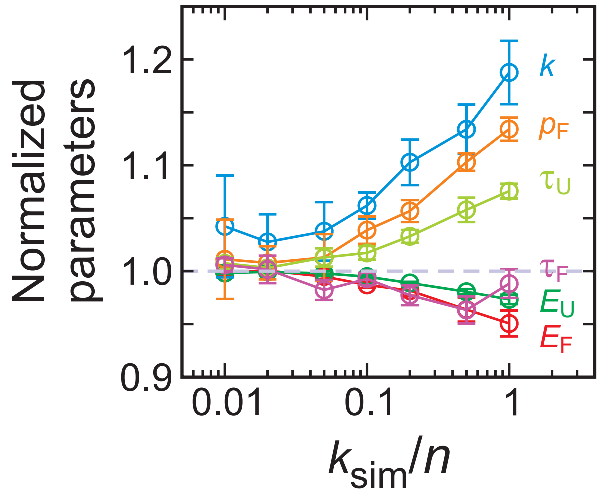

Figure S1. Maximum likelihood analysis of the photon trajectories with donor delay times simulated using the Gaussian chain model for the unfolded state. The parameters are normalized to the exact (input) values. The trajectories were simulated using a two-state model in Figure 2(c) with the parameters as in Figure 10 (E_U = 0.5, E_F = 0.85, p_F = 0.5, n = 50 ms^{-1}). The donor delay times in the folded state were generated using a single-exponential distribution with the mean delay time τ_{DF}/τ_D^0 = 0.15. The donor delay times in the unfolded state were generated using the distribution in eq 11 with $n_{Ds} = n(1 - E(r_s))$, $E(r_s) = 1/(1 + r_s^6)$, where r_s is the discretized donor-acceptor distance normalized to the Förster radius, and the distribution of the end-to-end distances is $p_s \propto r_s^2 \exp(-3r_s^2/2\langle r_s^2\rangle)$. The mean-square displacement is $\langle r_s^2\rangle^{1/2} = \langle r^2\rangle^{1/2}/R_0$ = 1.146, which corresponds to the FRET efficiency in the unfolded state E_U = 0.5. For simplicity, IRF, background noise and blinking were not considered in this simulation. Six parameters were extracted from 100 of 30 ms-long trajectories (150000 photons) using the likelihood function in eqs 8 - 10 with the single-exponential donor delay time distribution in both the folded and unfolded states, eq 13. The simulation was repeated 5 times for each value of k.

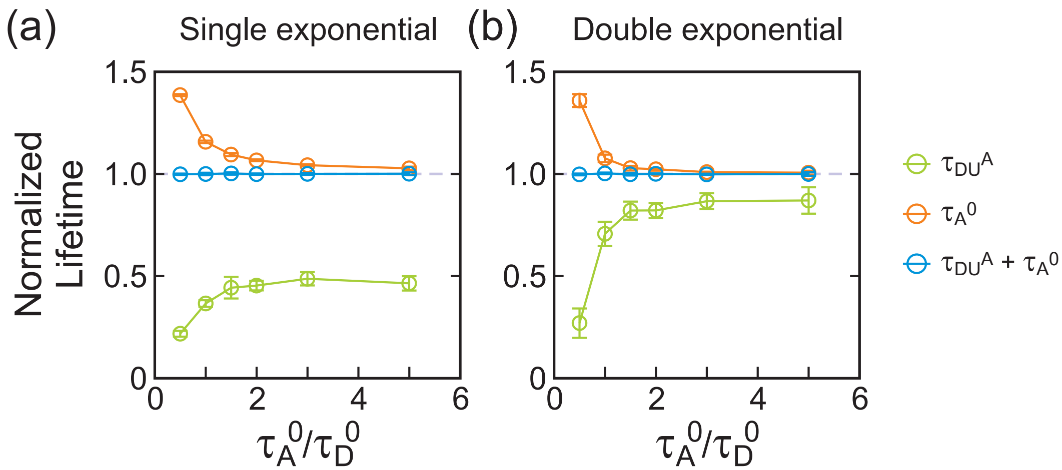

Figure S2. Donor (τ_{DU}^{A}, green) and acceptor (τ_{AU}^{0}, orange) excited state lifetimes in the unfolded state determined using the maximum likelihood analysis of the photon trajectories with acceptor delay times. The mean acceptor delay time (blue) is the sum of the donor and acceptor excited state lifetimes. The values of the extracted lifetimes are given relative to the simulated values. The photon trajectories were simulated as described in Figure S1 with k/n = 0.1. The acceptor delay times in the unfolded state were generated using the distribution in eq 12 with $n_{As} = nE(r_s)$ and $E(r_s)$ and p_s as in Figure S1 corresponding to the Gaussian chain model. In the folded state, the acceptor delay times were generated using the distribution in eq 14 with $\tau_{DF}^{A}/\tau_{D}^{0} = 0.15$. The acceptor excited state lifetimes (the same in the folded and unfolded states) are $\tau_{A}^{0}/\tau_{D}^{0}$ = 0.5, 1, 1.5, 2, 3, 5. The photon trajectories were analyzed using the likelihood functions in eqs 8 - 10 with $P_{DF}(\delta t) = P_{DU}(\delta t) = 1$, $P_{AF}(\delta t)$ in eq 14 and $P_{AU}(\delta t)$ in eq 14 (a) and eq 15 (b). The FRET efficiencies and the rate coefficients were fixed to the input values, and donor delay times were not analyzed. (a) Four lifetime parameters (τ_{DF}^{A}, τ_{AF}^{0}, τ_{DU}^{A}, τ_{AU}^{0}) were determined by maximizing the likelihood function. (b) Six parameters were determined (τ_{DU1}^{A}, τ_{DU2}^{A}, and α were determined instead of τ_{DU}^{A}). The mean lifetime in the unfolded state is calculated as $\tau_{DU}^{A} = \alpha\tau_{DU1}^{A} + (1 - \alpha)\tau_{DU2}^{A}$. For each value of τ_{A}^{0}, 5 sets of 100 30 ms-long trajectories were simulated (photon count rate n = 50 ms^{-1}). Each set was analyzed to extract four or six lifetime parameters.

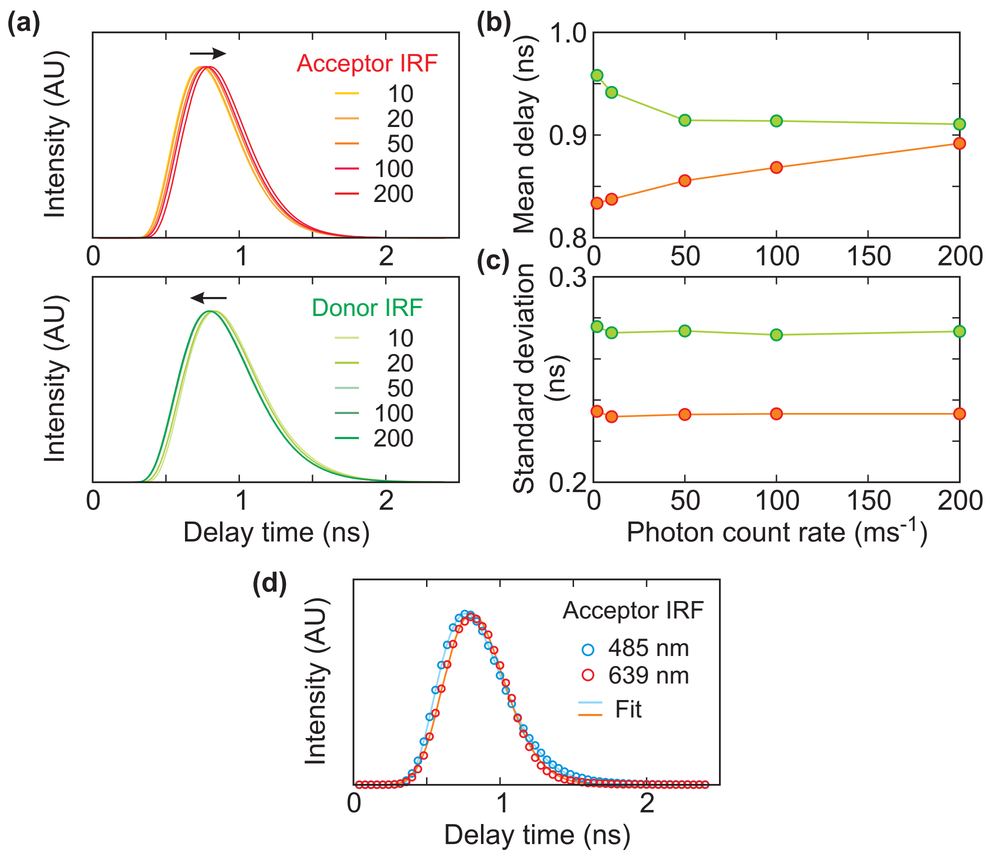

Figure S3. Photon count rate (a – c) and wavelength (d) dependence of IRF. (a) IRFs of the acceptor (upper) and donor (lower) channels were measured using the reflection of a 485 nm pulsed laser beam from a glass surface at various photon count rates (5 – 200 ms^{-1}). Only the fitted curves (Gamma distribution, eq 20) are shown for clarity. The mean delay time of the IRF ($\tau_{IRF}^{0} = t_0 + a/k_\gamma$) increases for the acceptor channel and decreases for the donor channel as the photon count rate increases (b), while their standard deviations ($\sqrt{a}/k_\gamma$, proportional to the width of the distribution) are insensitive to the photon count rate (c). These results show that only – the IRF offset (but not the shape) is affected by the photon count rate. The IRFs used in the analyses in the main text (Figure 2(e)) were measured at the photon count rate of ~ 10 ms^{-1}, which is lower than the average photon count rate of 50 – 100 ms^{-1} for the protein folding data. However, the analysis results would not be affected by these IRFs at different count rate because the offset t_0 is one of the fitting parameters in the analysis. The variation of the photon count rate over different molecules may slightly affect the results. The variation of the mean delay time is only 10 – 20 ps over the range of the count rate of 30 – 100 ms^{-1} in (b). (d) The standard deviation of the IRF in the acceptor channel measured by the reflection of a 639 nm beam (LDH-P-635, PicoQuant) is 0.210 ns, shorter than that by a 485 nm beam of 0.238 ns. Since the laser pulse width of the 639 nm laser (146 ps FWHM) is also shorter than that of the 485 nm laser (176 ps FWHM, manufacturer specification), the wavelength dependence of the IRF is supposed to be small.

Table S1. Lifetime parameters determined from the acceptor delay time analysis using the maximum likelihood method.[a]

		2-state/AD	2-state/AD2
α_3D	$\tau_{DF}{}^{A}/\tau_{D}{}^{0}$	0.059 (± 0.001)	0.059 (± 0.001)
	$\tau_{DU}{}^{A}/\tau_{D}{}^{0b}$	0.094 (± 0.002)	0.194 (± 0.016)
	$\tau_{AF}{}^{0}/\tau_{D}{}^{0}$	1.24 (± 0.004)	1.24 (± 0.004)
	$\tau_{AU}{}^{0}/\tau_{D}{}^{0}$	1.45 (± 0.005)	1.33 (± 0.019)
	$(\tau_{DU}{}^{A} + \tau_{AU}{}^{0})/\tau_{D}{}^{0}$	1.54 (± 0.005)	1.52 (± 0.005)
gpW	$\tau_{DF}{}^{A}/\tau_{D}{}^{0}$	0.092 (± 0.002)	0.092 (± 0.002)
	$\tau_{DU}{}^{A}/\tau_{D}{}^{0b}$	0.103 (± 0.002)	0.238 (± 0.017)
	$\tau_{AF}{}^{0}/\tau_{D}{}^{0}$	1.39 (± 0.004)	1.39 (± 0.004)
	$\tau_{AU}{}^{0}/\tau_{D}{}^{0}$	1.55 (± 0.006)	1.39 (± 0.020)
	$(\tau_{DU}{}^{A} + \tau_{AU}{}^{0})/\tau_{D}{}^{0}$	1.66 (± 0.005)	1.63 (± 0.005)
WW domain	$\tau_{DF}{}^{A}/\tau_{D}{}^{0}$	0.061 (± 0.003)	0.061 (± 0.002)
	$\tau_{DU}{}^{A}/\tau_{D}{}^{0b}$	0.079 (± 0.006)	0.215 (± 0.103)
	$\tau_{AF}{}^{0}/\tau_{D}{}^{0}$	1.35 (± 0.008)	1.35 (± 0.008)
	$\tau_{AU}{}^{0}/\tau_{D}{}^{0}$	1.48 (± 0.015)	1.33 (± 0.12)
	$(\tau_{DU}{}^{A} + \tau_{AU}{}^{0})/\tau_{D}{}^{0}$	1.56 (± 0.014)	1.54 (± 0.015)

[a] Errors are standard deviations obtained from the diagonal elements of the covariance matrix calculated from the likelihood function.

[b] In the models with a biexponential donor lifetime distribution (eq 15, 2-state/AD2, 4-state/AD2), $\tau_{DU}{}^{A} = \alpha\tau_{DU1}{}^{A} + (1 - \alpha)\tau_{DU2}{}^{A}$.

Table S2. Parameters determined simultaneously from the 2-state and 4-state analysis of color, arrival times, and donor and acceptor delay times.[a]

	α_3D		gpW		WW domain	
	2-state/ AD2s	4-state/ AD2s	2-state/ AD2s	4-state/ AD2s	2-state/ AD2s	4-state/ AD2s
E_F	0.901 (± 0.001)	0.916 (± 0.001)	0.816 (± 0.001)	0.853 (± 0.001)	0.790 (± 0.003)	0.827 (± 0.003)
E_U	0.551 (± 0.001)	0.563 (± 0.001)	0.481 (± 0.001)	0.506 (± 0.001)	0.484 (± 0.004)	0.518 (± 0.006)
k (ms^{-1})	0.823 (± 0.023)	0.792 (± 0.022)	2.94 (± 0.056)	2.78 (± 0.053)	13.06 (± 0.59)	8.01 (± 0.40)
p_F	0.458 (± 0.008)	0.508 (± 0.009)	0.425 (± 0.005)	0.449 (± 0.005)	0.523 (± 0.010)	0.655 (± 0.011)
k_b (ms^{-1})		1370 (± 220)		1670 (± 170)		1280 (± 150)
p_b^0		0.965 (± 0.002)		0.920 (± 0.002)		0.799 (± 0.006)
τ_{DF}/τ_D^0	0.238 (± 0.004)	0.162 (± 0.004)	0.373 (± 0.003)	0.241 (± 0.004)	0.259 (± 0.008)	0.186 (± 0.006)
τ_{DU}/τ_D^0	0.740 (± 0.003)	0.727 (± 0.003)	0.834 (± 0.003)	0.806 (± 0.003)	0.817 (± 0.007)	0.719 (± 0.011)
τ_{DF}^A/τ_D^0	0.059 (± 0.001)	0.059 (± 0.001)	0.092 (± 0.002)	0.093 (± 0.002)	0.061 (± 0.003)	0.063 (± 0.002)
τ_{DU}^A/τ_D^{0b}	0.193 (± 0.016)	0.197 (± 0.018)	0.238 (± 0.017)	0.244 (± 0.018)	0.182 (± 0.061)	0.254 (± 0.142)
τ_{AF}^0/τ_D^0	1.24 (± 0.004)	1.24 (± 0.004)	1.39 (± 0.004)	1.39 (± 0.004)	1.34 (± 0.009)	1.36 (± 0.008)
τ_{AU}^0/τ_D^0	1.33 (± 0.019)	1.33 (± 0.020)	1.39 (± 0.020)	1.39 (± 0.021)	1.35 (± 0.14)	1.30 (± 0.152)

[a] Errors are standard deviations obtained from the diagonal elements of the covariance matrix calculated from the likelihood function.

[b] $\tau_{DU}^A = \alpha\tau_{DU1}^A + (1 - \alpha)\tau_{DU2}^A$.