Supplementary Information

Molecular Dynamics Simulation of Energy Migration between Tryptophan Residues in Apoflavodoxin

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Provided as Supplementary Information are figures and tables of geometrical and dynamical parameters from molecular dynamics simulations, similarly as presented in the main text, but now obtained with the older force field FF99SBildn (see ref. 17 of the main text). The same numbering of figures and tables is maintained for easy comparison. Two extra figures are supplied. Fig. S11 provides experimental evidence from two single-tryptophan apoflavodoxin mutants that Trp167 has a larger absorption cross section at 300 nm than Trp74, which is the excitation wavelength. Fig. S12 shows the comparison between second order Legendre polynomial function, $P_2(t)$, of the three tryptophan residues obtained from two MD simulations with different force fields.

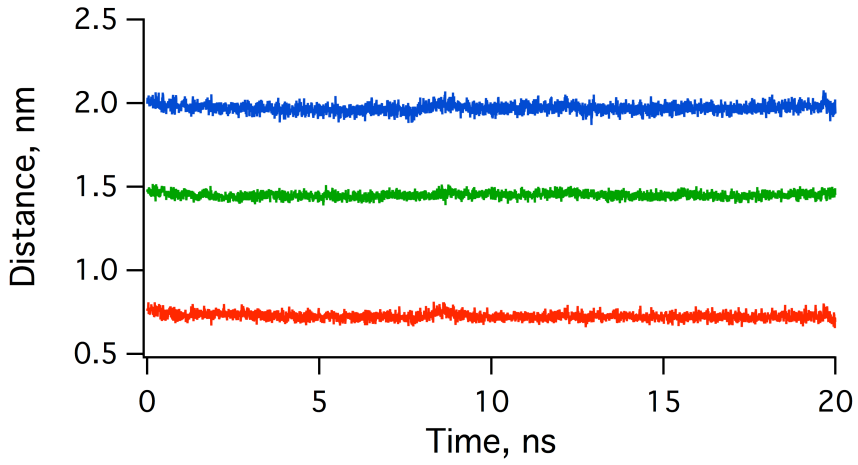

Figure S2. Fluctuating center-to-center distances between tryptophan pairs in apoflavodoxin. Green: Trp74-Trp167; blue: Trp74-Trp128; red: Trp167-Trp128. Average values are collected in Table S1.

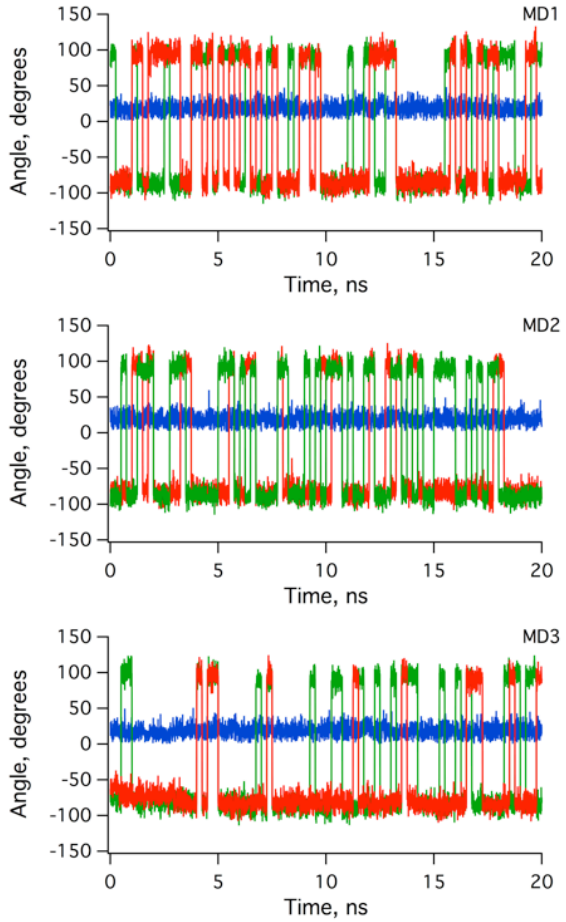

Figure S3. Fluctuating inter-planar (indole) angles between tryptophan pairs in three different MD simulations. Green: Trp74-Trp167; blue: Trp74-Trp128; red: Trp167-Trp128. For the three 20-ns trajectories of the inter-planar angle between Trp167 and Trp128 we counted on the average 12 events giving a flip-flop frequency of 0.6 $(ns)^{-1}$. Similarly, for the Trp74-Trp167 pair we counted 16 events yielding a flip-flop frequency of 0.8 $(ns)^{-1}$.

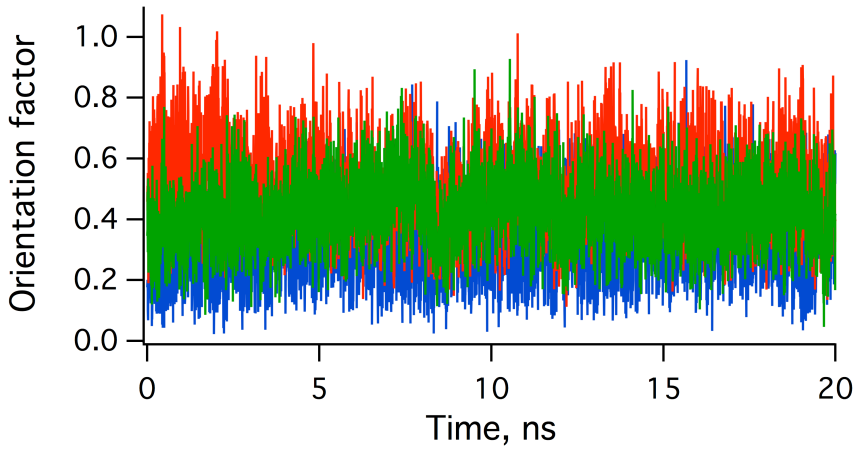

Figure S4. Fluctuating orientation factors κ^2 between two different tryptophan pairs in apoflavodoxin. Green: κ^2_{ac} ; blue: κ^2_{ab} ; red: κ^2_{cb} . The orientation factors are obtained using Eqs. 8-10 of the main text. Average values are collected in Table S1.

Table S1. Geometrical factors of tryptophan pairs in apoflavodoxin obtained from the MD trajectories

Trp pair*	Distance† (nm)	Inter-planar angle‡ (deg)	κ^2 (-)
cb	0.726 ± 0.022	-	0.51 ± 0.14
ac	1.450 ± 0.019	-	0.42 ± 0.12
ab	1.971 ± 0.026	18.5 ± 3.6	0.31 ± 0.13

**a*, *b* and *c* denote Trp74, Trp128 and Trp167, respectively.
†Distance is center-to-center distance.
‡Inter-planar angle is between the two indole rings of the pair. The inter-planar angle is not defined for *cb* and *ac*.

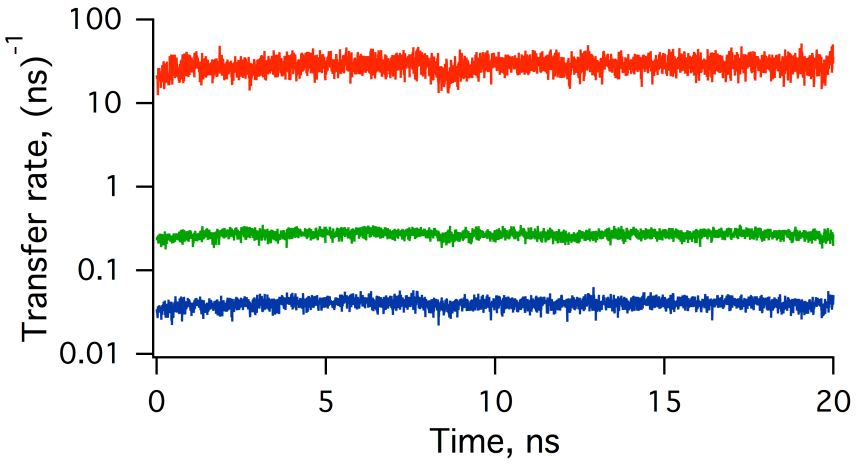

Figure S5. Trajectories of rate constants of energy transfer between two different tryptophan pairs in apoflavodoxin plotted on a semilogarithmic scale. FRET rate constants are obtained with Eqs. 11-13 of the main text. Green: Trp74-Trp167; blue: Trp74-Trp128; red: Trp167-Trp128. The FRET rate constants averaged over the MD trajectory are k_{cb} = 28.8 ± 5.5 $(ns)^{-1}$, k_{ac} = 0.270 ± 0.026 $(ns)^{-1}$ and k_{ab} = 0.040 ± 0.005 $(ns)^{-1}$.

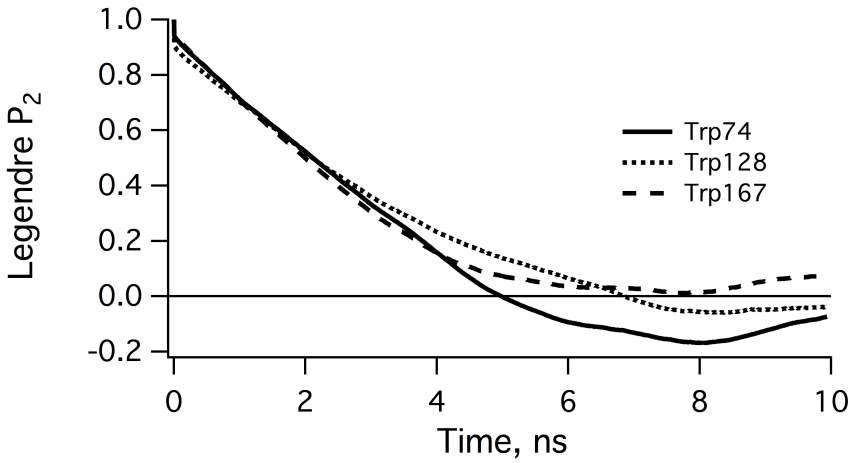

Figure S6. Second order Legendre polynomial function, $P_2(t)$, describing the reorientation of the emission transition dipole moment of the three tryptophan side chains in apoflavodoxin calculated with Eq. 7 (main text). Dotted line: Trp128; solid line: Trp74; dashed line: Trp167.

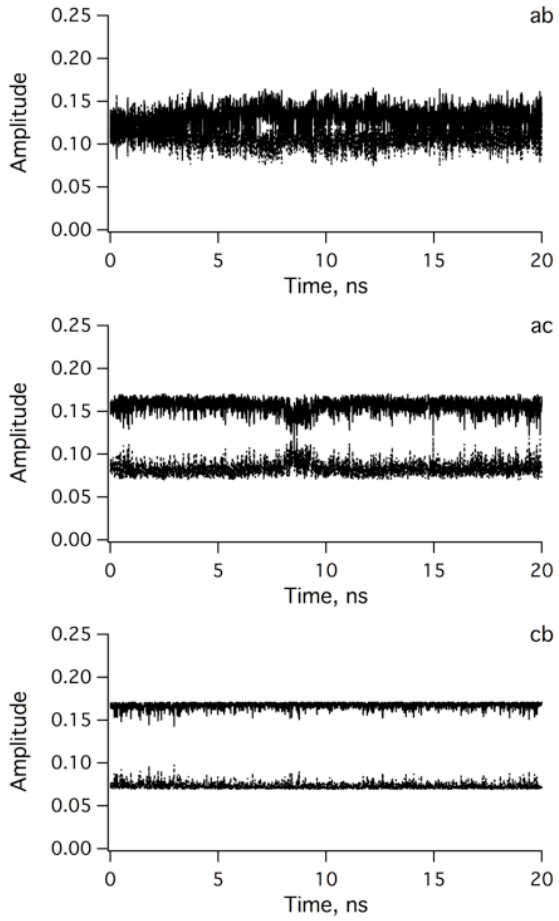

Figure S7: Time-dependence of fluctuating amplitudes β^T and β^R as obtained from the MD trajectory. The relevant pair is indicated above each panel. The solid traces are β^T. The dotted traces are β^R (in all panels these are the lower curves). The average values and standard deviations are collected in Table S2.

Table S2. Average values of the amplitudes β obtained from the MD trajectory

Trp pair	β^T (-)	β^R (-)
cb	0.168 ± 0.003	0.073 ± 0.003
ac	0.157 ± 0.008	0.083 ± 0.008
ab	0.131 ± 0.013	0.109 ± 0.013

*a, b and c denote Trp74, Trp128 and Trp167, respectively.
The sum of β^T and β^R is normalized to 0.24.

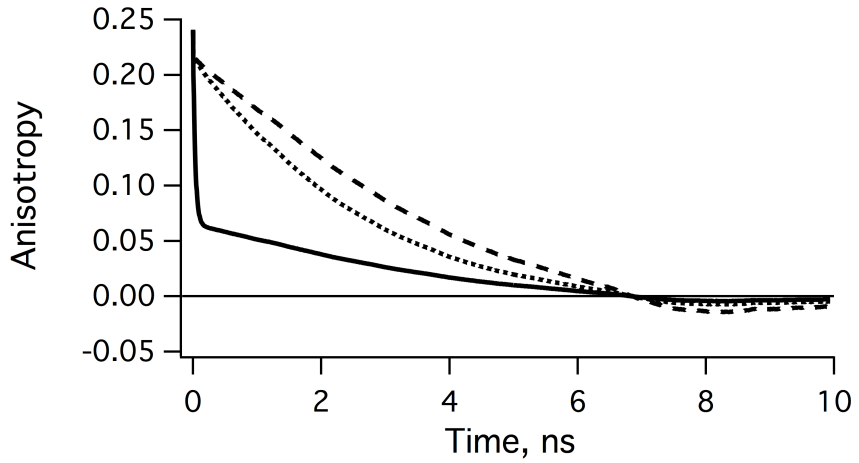

Figure S8. Normalized (to A_0 = 0.24) individual time-dependent anisotropy curves calculated from MD trajectories using Eqs. 14-16 of the main text. Dashed curve: emission from Trp128 upon excitation of Trp128 (A_{bb}); dotted curve: emission from Trp128 upon excitation of Trp74 (A_{ac}); solid curve: emission from Trp128 upon excitation of Trp167 (A_{cb}).

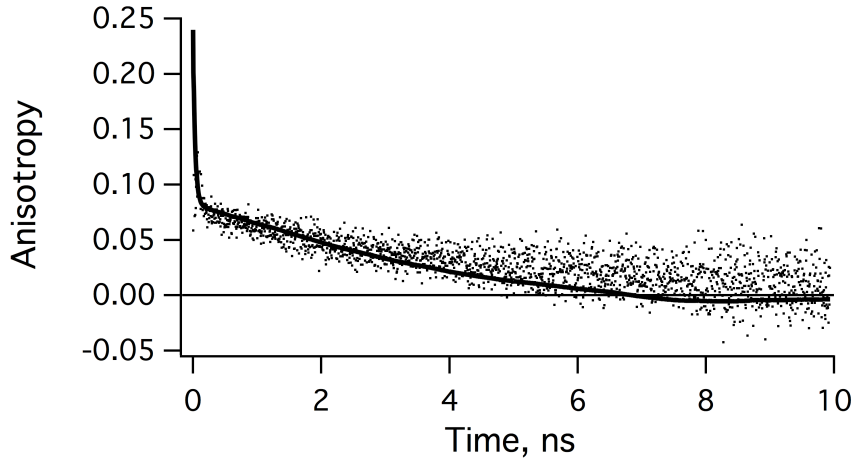

Figure S9. Experimental anisotropy (dots) and calculated anisotropy (solid line) consisting of a linear combination of individual curves in Fig. 8 with fractions f_i (i = 1 - 3) (Eq. 21 of main text) optimized in the fit procedure. Results are: f_1 = 0.12 (A_{bb}) , f_2 = 0.88 (A_{cb}) and f_3 = 0 (A_{ac}). Note that at times longer three nanoseconds the fitted anisotropy curve does not match the observed one.

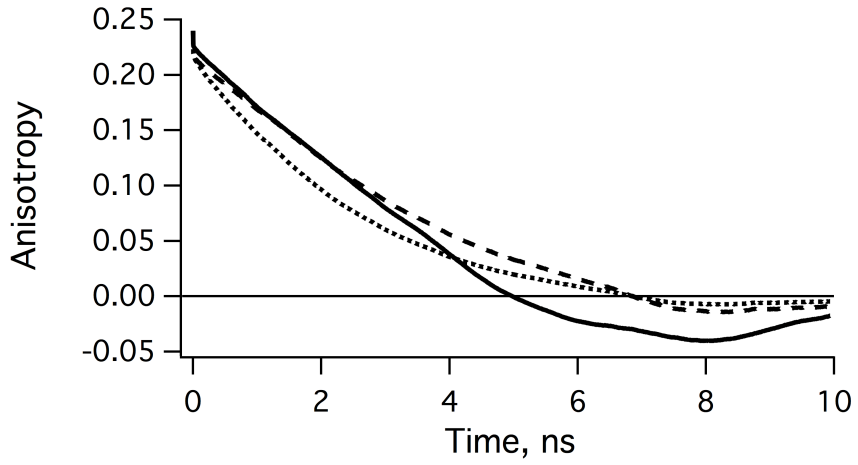

Figure S10. Normalized (to A_0 = 0.24), individual time-dependent anisotropy curves calculated from MD trajectories using Eqs. 14, 16 and 23 of the main text. Dashed curve: emission from Trp128 upon excitation of Trp128 (A_{bb}); dotted curve: emission from Trp128 upon excitation of Trp74 (A_{ac}); solid curve: emission from Trp74 upon excitation of Trp74 (A_{aa}).

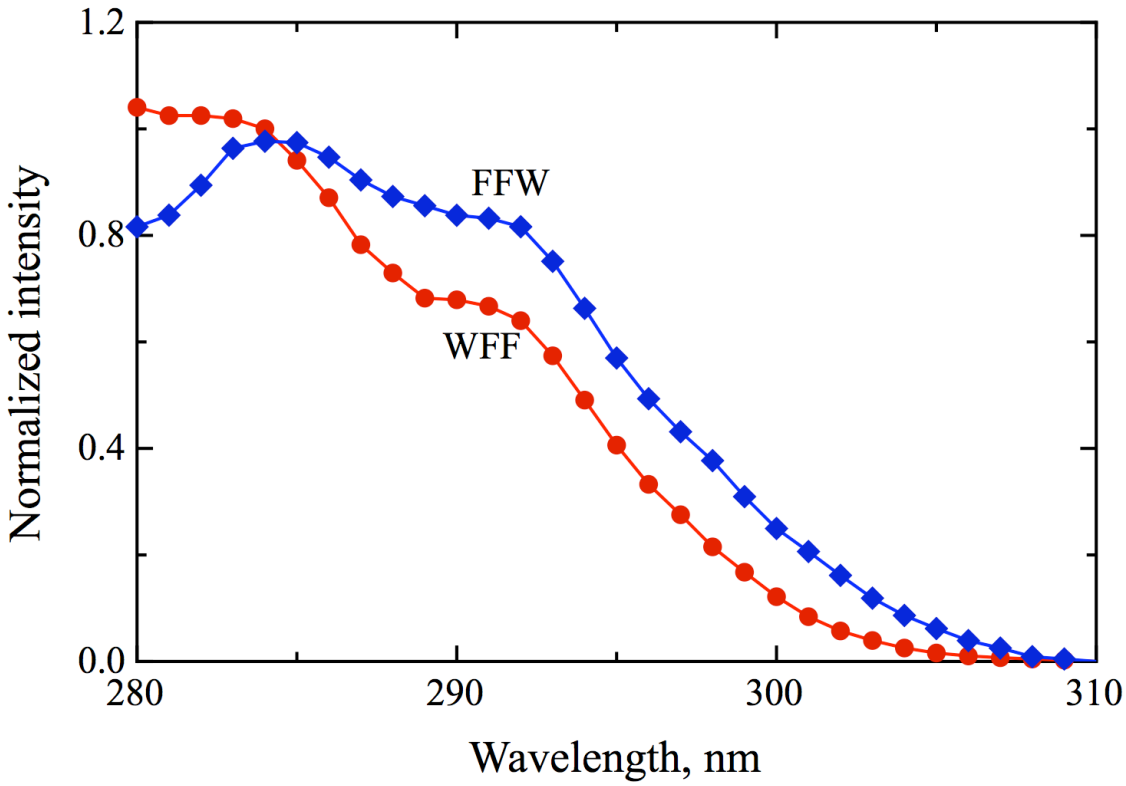

Figure S11. Corrected fluorescence excitation spectra of single-Trp apoflavodoxin mutants WFF (red filled circles) and FFW (blue diamonds) at detection wavelength of 350 nm. The spectra were obtained as described in ref. 7 (main text) and were normalized to unity at 284 nm.

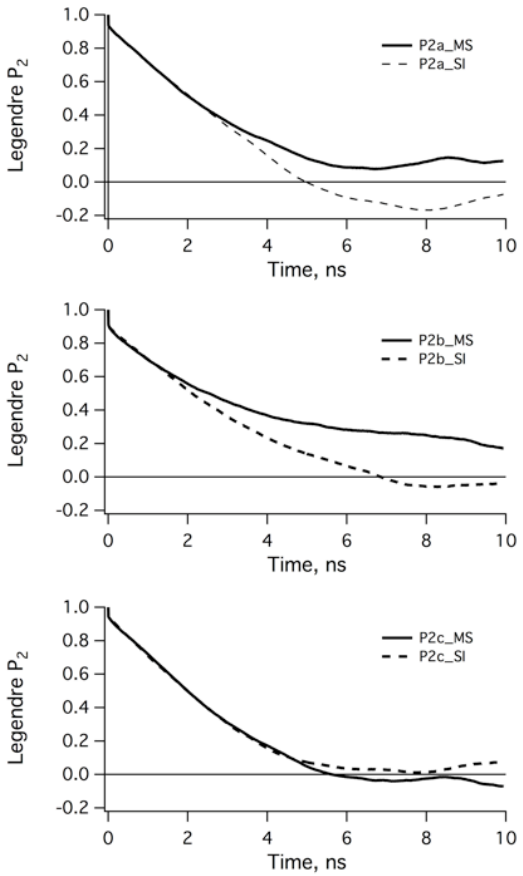

Figure S12. Comparison between second order Legendre polynomial function, $P_2(t)$, obtained from two MD simulations with different force fields. Solid line: data presented in manuscript (MS, FF03); dashed line: data presented in supplementary information (SI, FF99SBildn). a =Trp74, b = Trp128, c = Trp167.