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Concurrent presence of on- and off-pathway intermediates of apoflavodoxin at physiological ionic strength*

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Supplementary Information (SI)

Figure S1. In contrast to apoflavodoxin, tryptophan fluorescence emission of F44Y apoflavodoxin is sensitive to salt concentration. The emission spectra obtained at 100 mM KPPi (blue) and 10 mM KPPi (red) are normalized to the emission obtained at 100 mM KPPi. A, the emission spectrum of apoflavodoxin at 10 mM KPPi is identical to the corresponding spectrum at 100 mM KPPi. The tryptophans remain in a similar micro-environment and are not solvent exposed, because the protein is native at both salt concentrations. B, Decreasing salt concentration in case of F44Y apoflavodoxin leads to a reduction in fluorescence emission. This observation is due to formation of MG_{off} at low salt concentration. The tryptophans in MG_{off} are exposed to the solvent and have increased flexibility as compared to native apo-protein. Concentrations of apoflavodoxin and F44Y apoflavodoxin are 11.2 µM and 5.7 µM, respectively. Spectra are recorded at 25 °C and the pH is 6.0.

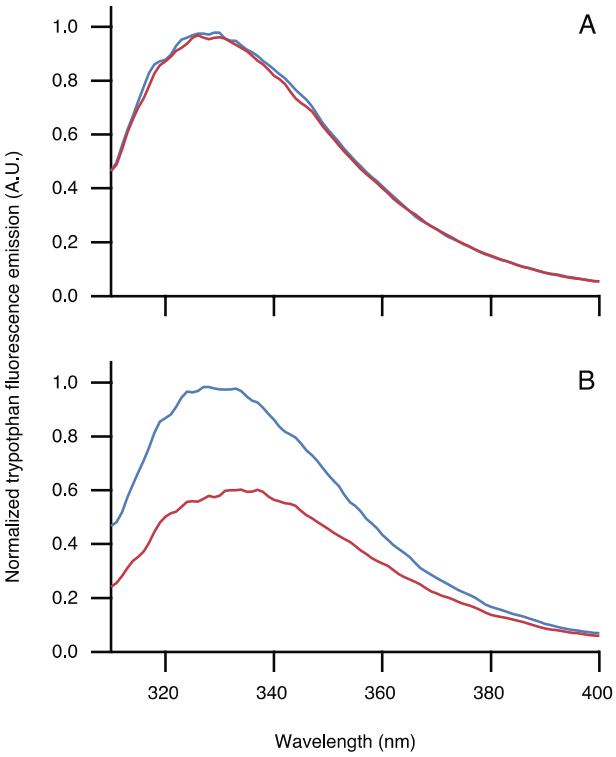

Figure S2. Fluorescence anisotropy decay (grey) and associated fit (black) obtained for 2 μM F44Y apoflavodoxin in 100 mM KPPi, pH 6.0, 25 ° C. The excitation wavelength is 300 nm and the emission is measured at 348.8 nm. The optimized parameters (Eq. 15) are: ϕ_{T1} = 0.07 ns (fixed) (β_{T1} = 0.05), ϕ_{T2} = 2.6 ns (β_{T2} = 0.07) and ϕ_r = 12.7 ns (β_r = 0.06), the goodness-of-fit parameter χ^2 = 1.30.

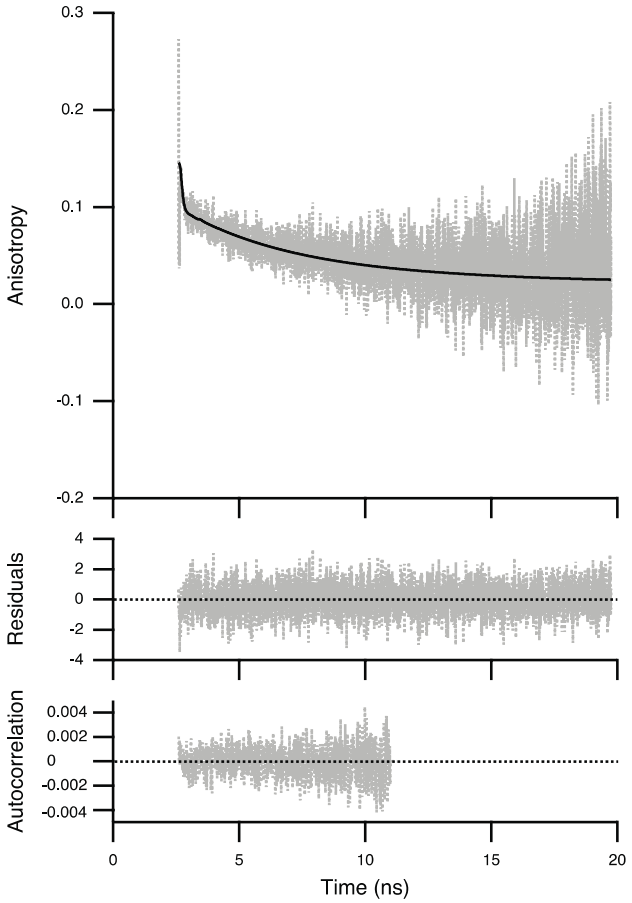

Figure S3. KPPi-concentration dependent, steady-state tryptophan fluorescence anisotropy (R) of F44Y apoflavodoxin. Comparison between steady-state fluorescence anisotropy as measured by Nabuurs *et al*. on a Fluorolog 3.2.2 fluorometer (blue circles [19]) and values derived in this study from time-resolved anisotropy measurements using a time-correlated single photon counting device (green diamonds). As the anisotropies are recorded on set-ups with differences in excitation/emission wavelengths, the absolute anisotropy values differ. Upon correcting time-resolved data for this difference in offset (red triangles), both data sets overlap.

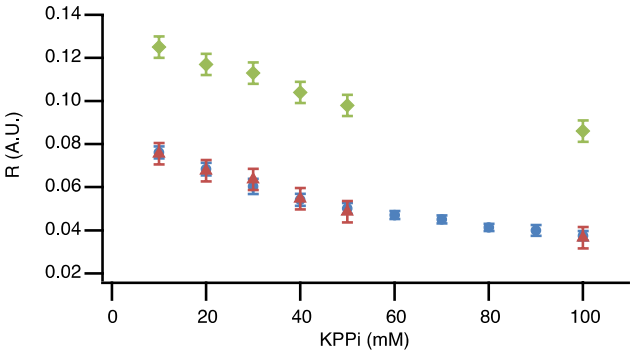

Figure S4. Average fluorescence lifetime <τ> of F44Y apoflavodoxin as a function of KPPi concentration. The data have been fitted to a sigmoidal curve, as the (un)folding of MG_{off} has a sigmoidal transition, as shown by single-molecule FRET [35]. Data points are taken from Table 2.