# Excited State Dynamics Can Be Used to Probe Donor-Acceptor Distances for H-Tunneling Reactions Catalysed by Flavoproteins

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**SUPPORTING MATERIAL**

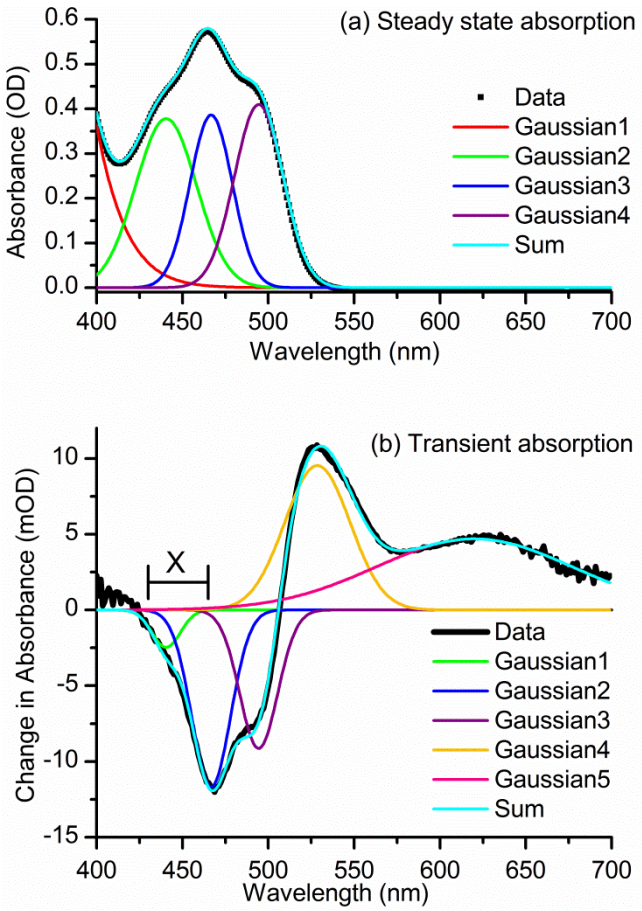


**Figure S1.** a) Steady state absorption spectra of wild-type MR fitted with 4 gaussian functions centred at 245 (Gaussian1), 441(Gaussian2), 467(Gaussian3) and 494(Gaussian4) nm. b) Transient absorption spectra of wild-type MR recorded 1 ps after excitation at 375, fitted with 5 gaussian functions, positions of Gaussians 1-3 fixed to be those of Gaussians 2-4 in fit of steady state spectra, corresponding to bleach of the ground state. Gaussians 4 and 5 centred at 529 and 620 nm respectively are due to excited state absorption. The rate of ground state recovery is calculated from average of data within range marked X (430 – 465 nm), where no significant contribution from Gaussians 4 and 5 (excited state absorption) is observed.

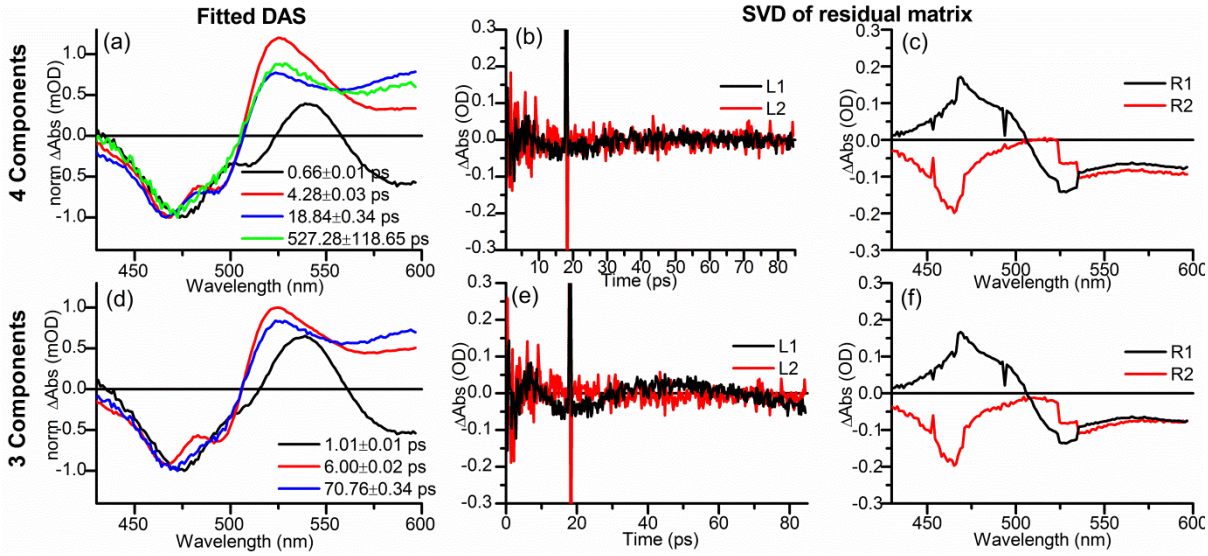

**Figure S2.** Decay associated difference spectra from global analysis of transient absorption data of wild-type MR. a) 4 component fit and associated lifetimes and d) 3 component fit with associated lifetimes. The residual matrix from the fit is then deconvolved by singular value decomposition to the component times (left, (b) and (e)) and wavelengths (right, (c) and (f)). The lack of obvious structure in the time-component of the 4-component fit residual implies a good fit has been obtained.

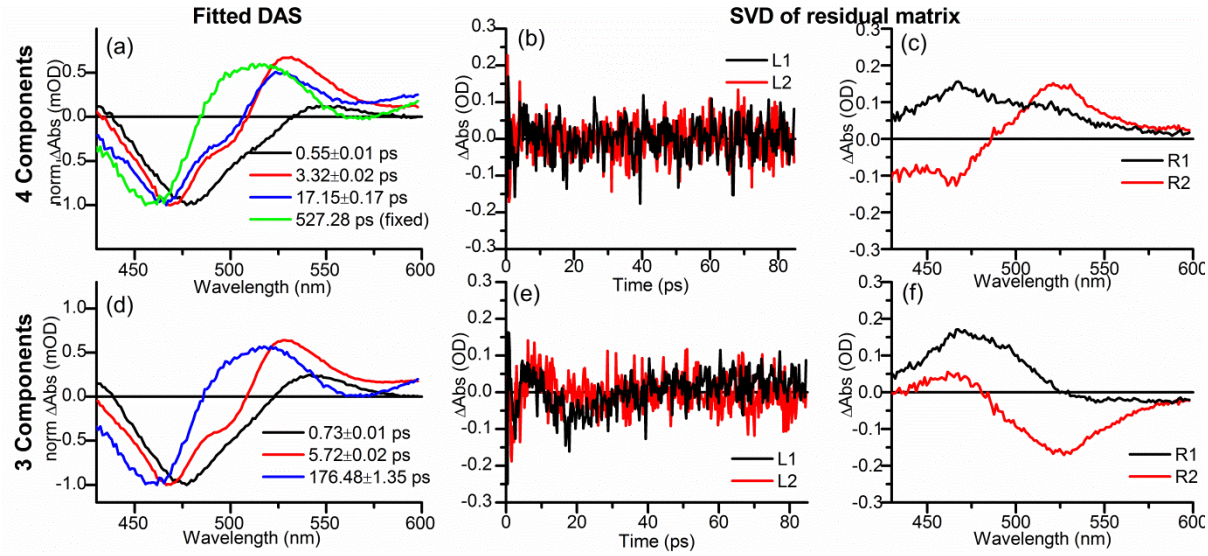


**Figure S3.** Decay associated difference spectra from global analysis of transient absorption data of wild-type MR with $NADH_4$. a) 4 component fit and associated lifetimes (long component fixed to ensure convergence), and d) 3 component fit with associated lifetimes. The residual matrix from the fit is then deconvolved by singular value decomposition to the component times (left, (b) and (e)) and wavelengths (right, (c) and (f)). The lack of obvious structure in the time-component of the 4-component fit residual implies a good fit has been obtained.

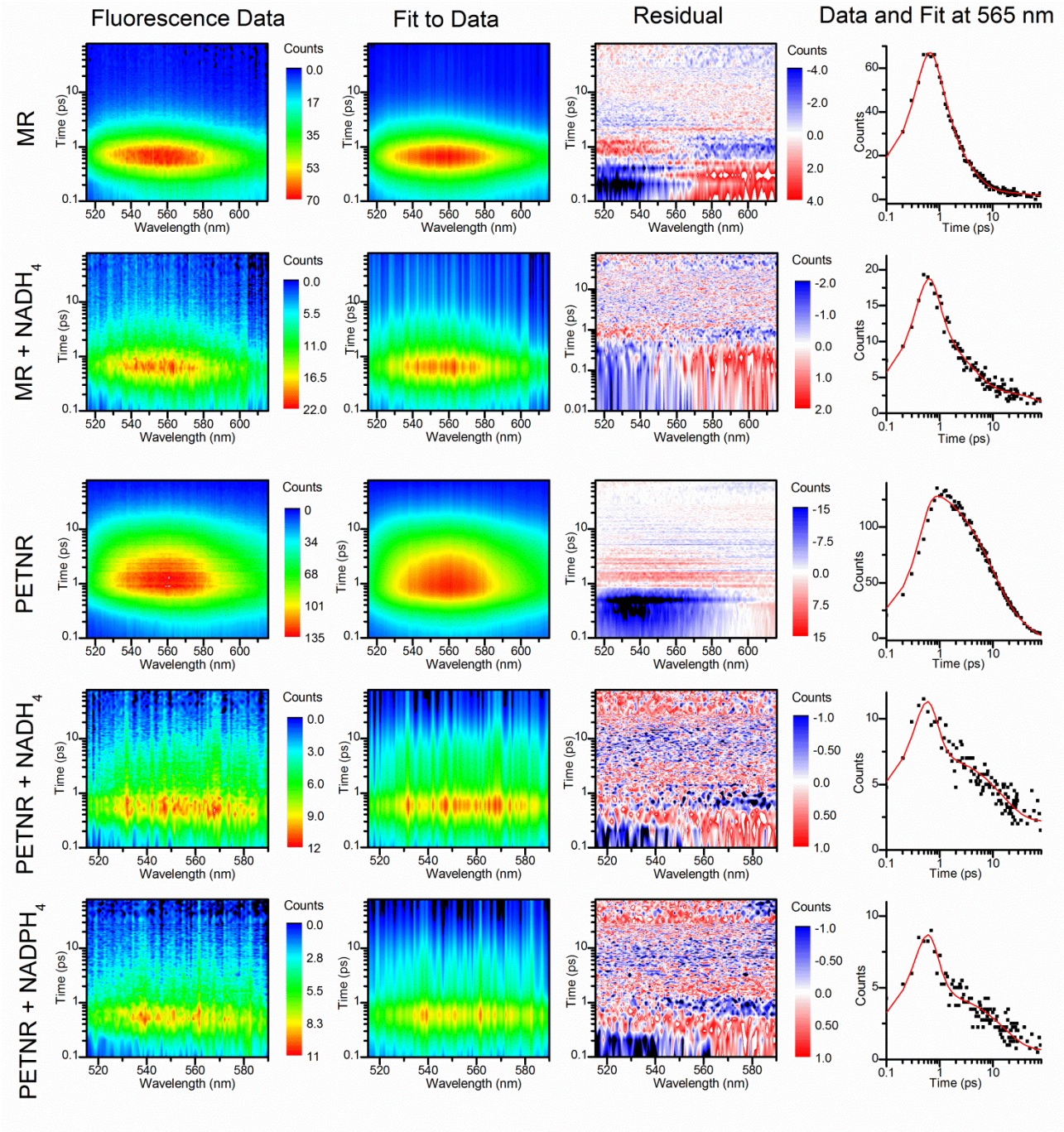


**Figure S4.** Fluorescence up-conversion data, results of fitting procedure, and residuals from fitting procedure, for wild-type MR, wild-type MR bound to $NADH_4$, PETNR, PETNR bound to $NADH_4$, and PETNR bound to $NADPH_4$. Experiments were carried out using the laser system described in the main text and a commercial Halcyone (Ultrafast Systems LLC) fluorescence up-conversion spectrometer with CCD detector. Samples were excited at 375 nm with powers of ~0.6 uJ, data were collected over 80 – 105 minute time frames. Data analysis was carried out using in house software, data was globally fitted to multiexponential functions convoluted with a Gaussian function with a width of 0.5 ps to represent the instrument response function, time zero was fixed at 0.3 ps.

| | $\tau_1$ (ps) relative amplitude | $\tau_2$ (ps) relative amplitude | $\tau_3$ (ps) relative amplitude | $k_{S1}$ ($ps^{-1}$) | $k_{ET}$ ($ps^{-1}$) |
|---|---|---|---|---|---|
| wt MR | 0.73±0.01<br>58±8% | 3.3±0.1<br>31±4% | 83.3±2.7<br>11±2% | 0.69±0.17 | 0.21±0.33 |
| wt MR - $NADH_4$ | 0.44±0.01<br>62±6% | 3.6±0.1<br>23±2% | 107±4<br>15±2% | 0.90±0.28 | |
| PETNR | - | 8.4±0.1<br>61±4% | 35.7±0.6<br>39±3% | 0.05±0.01 | |
| PETNR - $NADH_4$ | 0.32±0.01<br>83±7% | - | 15.2±0.1<br>17±1% | 0.35±0.06 | 0.29±0.05 |
| PETNR - $NADPH_4$ | 0.37±0.01<br>81±6% | - | 16.2±0.2<br>19±1% | 0.30±0.04 | 0.29±0.04 |

**Table S1.** Kinetic fit parameters and calculated rates of excited state population loss, $k_{S1}$, and photoinduced electron transfer rates for fluorescence data shown in figure S4, for wild-type MR, wild-type MR bound to $NADH_4$, PETNR, PETNR bound to $NADH_4$, and PETNR bound to $NADPH_4$. Errors quoted on the lifetimes and relative amplitudes are those from the fitting procedure, it should be noted that the time resolution of the system is on the order of 500 fs, and this is the value used for error propagation to the rate values if the error on the fit is smaller than this. Data analysis was carried out using in house software, data was globally fitted to multiexponential functions convoluted with a Gaussian function with a width of 0.5 ps to represent the instrument response function, time zero was fixed at 0.3 ps. Calculation of rates of $k_{S1}$ and photo-induced electron transfer , $k_{ET,}$ were carried out as described in the main manuscript, exponential components with lifetimes greater than 100 ps were not used in this calculation as they are not likely to include charge-transfer any contribution.