

## Internal electron transfer in multi-site redox enzymes is accessed by laser excitation of thiouredopyrene-3,6,8-trisulfonate (TUPS)

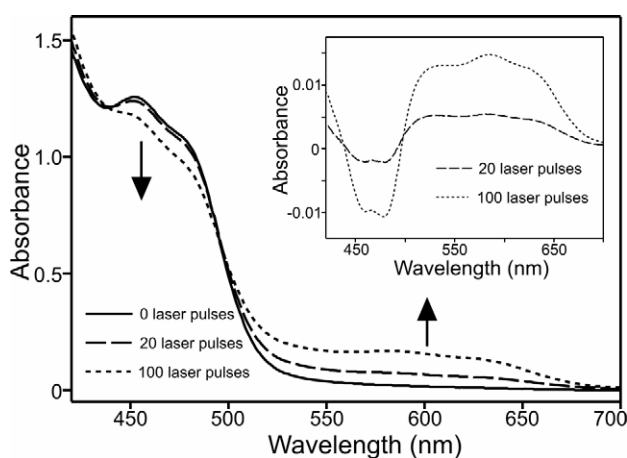
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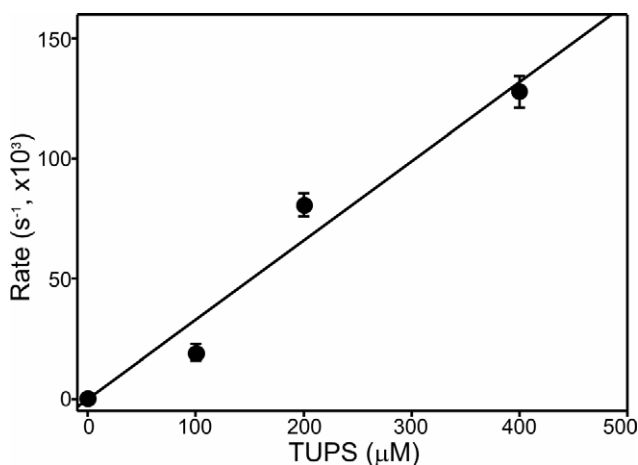
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### Supplementary figures

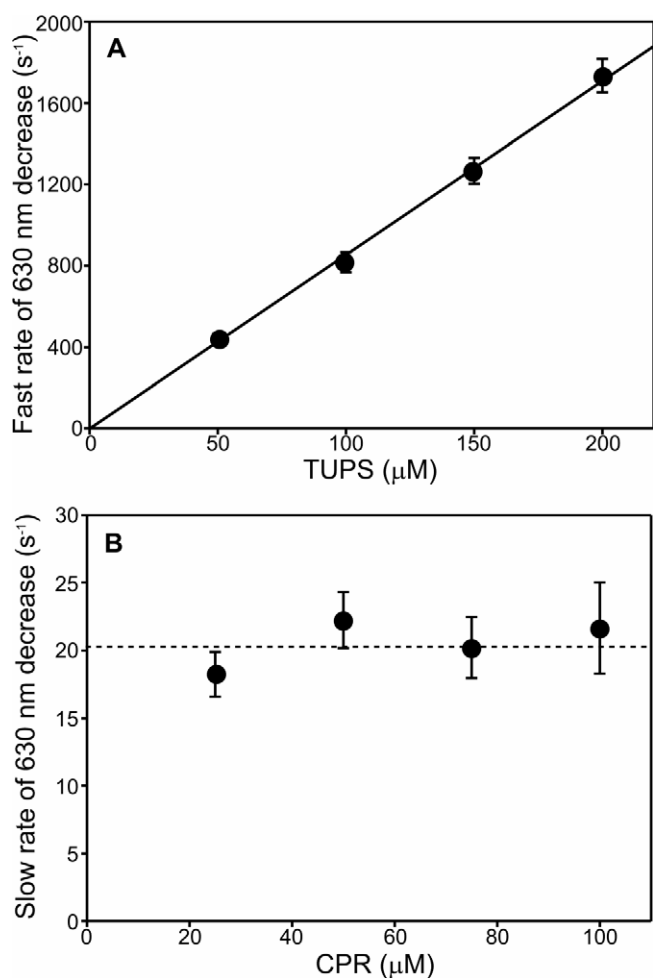
**Figure S1** Absorbance spectra of 50  $\mu\text{M}$  CPR in the presence of 200  $\mu\text{M}$  TUPS before laser photoexcitation and after excitation with 20 and 100 laser pulses at 355 nm. The inset shows the absorbance difference spectra, using a non-excited sample as a blank.



**Figure S2** The rate of electron transfer from photoexcited TUPS to CPR measured at a range of TUPS concentrations. The error bars were calculated from the average of at least 5 transients.



**Figure S3.** Dependence of slower kinetic phases on the concentration of TUPS and CPR. **(A)** The rate of the initial phase of decrease in absorbance at 630 nm measured over a range of TUPS concentrations. The error bars were calculated from the average of at least 5 transients. **(B)** The rate of the slower phase of decrease in absorbance at 630 nm measured over a range of CPR concentrations. The error bars were calculated from the average of at least 5 transients.



**Figure S4** Absorbance spectra of TUPS-nNOS reductase sample after covalent attachment to the protein as described in the *Experimental* section. The TUPS and nNOS reductase absorption peaks are indicated.

