Supporting Information for:

Solvent-slaved proteins motions accompany proton coupled electron transfer catalysed by copper nitrite reductase

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Experimental Details:

All reagents were of analytical grade and were purchased from Sigma-Aldrich, unless otherwise stated. *Alcaligenese xylosoxidans* copper nitrite reductase (*Ax*NiR) was expressed and purified as previously described.[1] pH-jump measurements were performed in MTEN buffer (50 mM MES, 25 mM Tris, 25 mM ethanolamine and 100 mM NaCl) containing 20 mM potassium nitrite to maintain the ionic strength across the pH range used. Ascorbate and phenazinium methyl sulphate (PMS) were used to selectively reduce the T1Cu at pH 9, as previously described,[2,3] and all samples were passed down desalting columns to remove surplus reductant prior to any pH-jump experiments. Stopped-flow measurements were performed at 4 ^{o}C using an Applied Photophysics SX10 instrument housed within an anaerobic glovebox (< 5ppm) with ~20 μM *Ax*NiR (final concentration). 3-4 repeats were made in each stopped-flow measurement and are represented as an average ± one standard deviation. High-pressure stopped-flow measurements were measured under anaerobic conditions using a Hi-Tech Scientific HPSF-56 stopped-flow instrument. MTEN buffer was used for high-pressure stopped-flow studies as all the buffering components have low volume of ionisation values,[4] minimising the influence of pressure on solution pH.

Gamma-radiolysis studies were performed in 50 mM potassium phosphate buffer (pH 7.0) containing 100 mM of tert-butanol and 50 mM N-methyl nicotinamide using a

Model 812 ^{60}Co-source as the cryo-reductant at 80 K. All EPR samples were measured on a Bruker ELEXYSYS-500/580 X-band EPR spectrometer at 20 K using a microwave power of 36 dB, a modulation amplitude of 5 G, a sweep time of 84 s and an average microwave frequency of 9.382 GHz.

Tables:

Table S1. High-pressure stopped-flow fit parameters for the analysis of observed rate constants for PCET in *Ax*NiR.

k_0 / s^{-1}	303 ± 23
$\Delta V^{\ddagger}$ / $cm^3\,mol^{-1}$	9.3± 4.8
$\Delta B^{\ddagger}$ / $cm^3\,mol^{-1}\,kbar^{-1}$	-16.8 ± 6.9

Table S2 Observed rate constants *vs*. pressure for PCET reaction catalysed by *Ax*NiR

Pressure (Pa)	k_{obs} (s^{-1})	SD
1	378	35
250	258	11
500	225	4
750	183	2
1000	152	17
1250	103	8
1500	66	10

Table S3. Solvent viscosity stopped-flow fit parameters for the analysis of observed rate constants for PCET in *Ax*NiR.

σ / cP	5.31 ± 2.35
$\Delta G^{\ddagger}$ / kJ mol^{-1} (277.15 K)	53.6 ± 0.3

Table S4 Observed rate constants *vs*. solvent viscosity for PCET reaction catalysed by *Ax*NiR

Viscosity (cP)	k_{obs} (s^{-1})	SD
1.55	377	35
2.09	384	35
2.925	353	27
4.315	313	9
6.81	205	16

Figures:

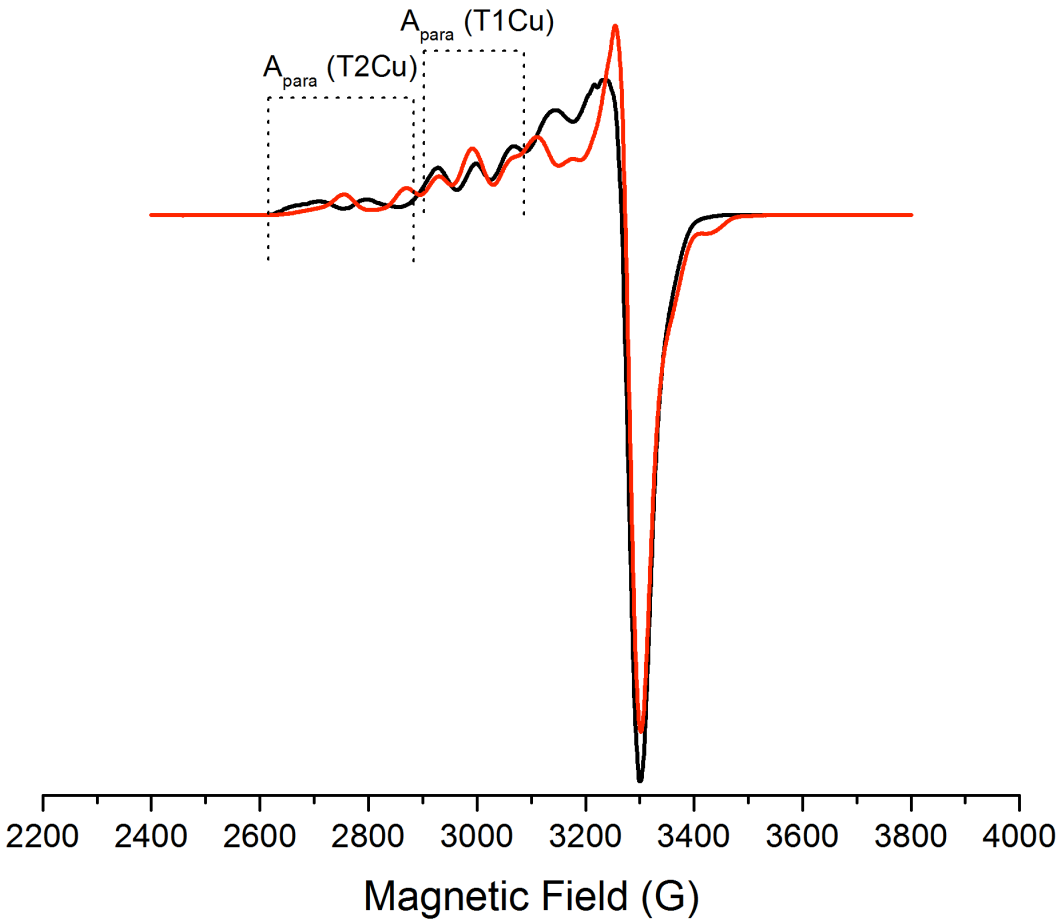

Figure S1. cw-EPR spectra of nitrite-bound (red) and nitrite-free (black) *Ax*NiR, measured as a frozen solution at 20 K. The weak parallel features arise from ^{63,65}Cu nuclei of T1 and T2Cu centres and the effect of substrate binding are indicated by the black, dotted goal posts.

References:

1 S. Brenner, D. J. Heyes, S. Hay, M. A. Hough, R. R. Eady, S. S. Hasnain and N. S. Scrutton, *J. Biol. Chem.*, 2009, **284**, 25973–25983.

2 N. G. H. Leferink, R. R. Eady, S. S. Hasnain and N. S. Scrutton, *FEBS J.*, 2012, **279**, 2174–2181.

3 S. Ghosh, A. Dey, Y. Sun, C. P. Scholes and E. I. Solomon, *J. Am. Chem. Soc.*, 2009, **131**, 277–288.

4 Y. Kitamura and T. Itoh, *J. Solution Chem.*, 1987, **16**, 715–725.