Supporting Information to

Spectroelectrochemical Investigation of Intramolecular and Interfacial Electron-Transfer Rates Reveals Differences Between Nitrite Reductase at Rest and During Turnover

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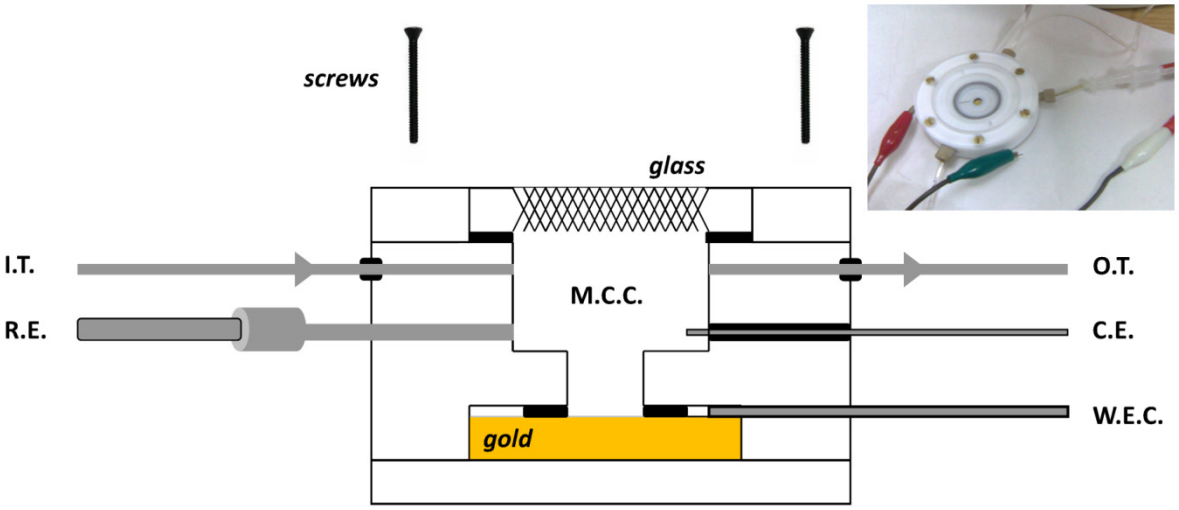

Figure 8: A schematic presentation (cross-section, side-view) of the used home-built spectro-electrochemical cell. The spectro-electrochemical cell is assembled using screws and o-ring rubbers (black) to get a proper seal and prevent electrolyte leakage from a main cell compartment (M.C.C.), into which three electrodes are fitted: gold working electrode connected to the potentiostat via the copper wire (W.E.C.), platinum counter electrode (C.E.) and silver/silver chloride reference electrode (R.E.). The buffer/chemicals flow into the cell via inlet tubing (I.T.) and come out via outlet tubing (O.T.). The cell is top-closed with a glass slide enabling for epi-fluorescent measurement during the electrochemistry applied. The drawing is not to scale. The distance between the glass coverslip and gold working electrode is approximately 5 mm. (Insert) A photo of the home-build cell.

pH	k_{-1}^{A} (s^{-1})	k_{-1}^{B} (s^{-1})	$k_{ET}^{1/3}$ (s^{-1})	$k_{ET}^{2/3}$ (s^{-1})	k_1^{A} (s^{-1})	k_1^{B} (s^{-1})	k_3 (s^{-1})	K_D^{A} (mM)	K_D^{B} (mM)	TSS (A.U.)
5.45	298.3	11.7	93.4	3.9	2100.0	11.7	120.1	0.087	0.65	10.51
	298.3	**0.0**	93.5	3.9	2100.0	11.6	119.8	0.086	0.61	10.47
	298.3	**20.0**	93.4	3.9	2100.0	12.7	120.9	0.087	0.66	10.53
	298.3	**50.0**	93.1	3.9	2100.0	14.2	122.6	0.089	0.75	10.61
	298.3	**100.0**	92.8	3.9	2100.0	16.6	125.3	0.091	0.89	10.67
	0.0	10.4	94.0	4.1	2100.0	10.4	115.3	0.088	0.65	12.43
	1000.0	13.9	92.5	3.7	2100.0	13.9	131.3	0.083	0.63	6.96
	2100.0	16.0	91.7	3.5	2100.0	16.0	146.0	0.075	0.62	4.72
	3000.0	16.9	91.6	3.4	2100.0	16.9	154.8	0.067	0.61	4.16
	4100.0	17.6	91.6	3.3	2100.0	17.6	161.7	0.058	0.61	3.95
	28.41	-1.5	96.3	3.5	**200.0**	-1.5	1039.8	0.833	8.68	11.33
	56.8	10.4	92.5	3.8	**400.0**	10.4	202.4	0.153	3.41	10.37
	142.0	11.5	93.1	3.9	**1000.0**	11.5	132.9	0.096	1.35	10.47
	710.2	11.7	93.5	3.9	**5000.0**	11.7	116.6	0.084	0.27	10.52
6.85	24.4	49.6	63.2	3.1	24.4	49.6	113.4	0.49	4.64	4.47
	2100	52.3	63.3	2.6	2100	52.3	164.3	0.93	0.070	11.88
	24.4	48.7	63.3	3.1	24.2	48.7	121.0	0.53	5.03	4.46
	0.0	46.0	53.9	3.8	16.4	46.0	113.4	0.58	1.83	8.10
	50.0	50.2	69.4	2.8	26.6	50.2	113.4	0.40	5.72	3.53
	100.0	50.6	80.4	2.3	29.1	50.6	113.4	0.29	7.64	2.86
	19.9	**0.0**	64.6	3.3	19.9	38.2	113.4	0.48	5.67	3.97
	28.9	**100.0**	62.3	3.1	28.9	61.6	113.4	0.51	4.22	4.87
	37.4	**200.0**	61.6	3.0	37.4	85.0	113.4	0.51	3.67	5.44

Table 3: two possible scenarios of NiR enzyme catalysis at pH 6.85, as well as the minor role of backward, intramolecular ET rates (k_{-1}^{A} and k_{-1}^{B}) at both pH (5.45 and 6.85). The underlined rate constants are fixed values as given in Table 1 of the manuscript (gray). The rate constants in bold stand for new variations of those fixed parameters. At high pH (6.85), the enzyme changes form route A to route B when $K_D^{A} < K_D^{B}$ and $k_1^{A} < k_1^{B}$ (red areas) or from route B to route A when $K_D^{A} > K_D^{B}$ and $k_1^{A} > k_1^{B}$ (blue areas). At low pH (5.45), the red areas indicate unrealistic ET rate values when trying to force the enzyme catalysis to start from route B and change to route A at high nitrite concentration. It can be concluded that NiR can never be described by following relations $K_D^{A} > K_D^{B}$ and $k_1^{A} < k_1^{B}$. Therefore, the only possibility for the enzyme at pH 5.45 is to switch from route A (at low nitrite) to route B (at high nitrite), as given by relations $K_D^{A} < K_D^{B}$ and $k_1^{A} > k_1^{B}$ (gray areas). TSS stands for total sum of squares and signifies a difference between raw and modeled data.

The following (modeled) equations for k_{cat} and F_i were used to fit the data obtained from spectroelectrochemistry experiments.

$$k_{cat} = (S^2 k_3^2 k_{ET} K_D^B k_1^A + S k_3 k_{ET}^2 K_D^B k_1^A K_D^A + S^2 k_3 k_{ET}^2 k_1^A K_D^B + S^3 k_3 k_{ET}^2 k_1^B + S^3 k_3^2 k_{ET} k_1^B + S^2 k_3 k_{ET}^2 k_1^B K_D^A) / (k_{ET} S^2 k_3 k_1^A K_D^B + 2 K_D^B S k_1^A k_{ET}^2 K_D^A + K_D^B S^2 k_3^2 k_{ET} + K_D^B S^2 k_3 k_{ET} k_{-1}^B + K_D^B S^2 k_3^2 k_1^A + K_D^B S k_3 k_{ET} k_{-1}^A K_D^A + S^2 k_3 k_{ET}^2 K_D^B + k_{ET}^2 S^2 k_1^A K_D^B + K_D^B S k_1^A k_{ET} k_3 K_D^A + K_D^B S k_3 k_{ET}^2 K_D^A + k_1^A k_{ET}^2 (K_D^A)^2 K_D^B + S^3 k_3 k_{ET}^2 + S^3 k_3^2 k_{ET} + S^3 k_3^2 k_1^B + k_{ET}^2 S^3 k_1^B + k_{ET} k_3 S^3 k_1^B + S^2 k_3 k_1^B k_{ET} K_D^A + S^2 k_3 k_{ET} k_{-1}^A K_D^A + k_{ET}^2 S k_1^B (K_D^A)^2 + S^2 k_3 k_{ET}^2 K_D^A + S^3 k_3 k_{-1}^B k_{ET} + 2 k_1^B k_{ET}^2 S^2 K_D^A)$$

$$F_i = (K_D^B S^2 k_3^2 k_{ET} + K_D^B S k_3 k_{ET}^2 K_D^A + k_1^A k_{ET}^2 (K_D^A)^2 K_D^B + k_{ET}^2 S^2 k_1^A K_D^B + 2 K_D^B S k_1^A k_{ET}^2 K_D^A + K_D^B S^2 k_3 k_{ET} k_{-1}^B + S^2 k_3 k_{ET}^2 K_D^B + K_D^B S k_3 k_{ET} k_{-1}^A K_D^A + k_{ET}^2 S^3 k_1^B + S^3 k_3 k_{ET}^2 + k_{ET}^2 S k_1^B (K_D^A)^2 + 2 k_1^B k_{ET}^2 S^2 K_D^A + S^3 k_3 k_{-1}^B k_{ET} + S^2 k_3 k_{ET}^2 K_D^A + S^2 k_3 k_{ET} k_{-1}^A K_D^A + S^3 k_3^2 k_{ET}) / (k_{ET} S^2 k_3 k_1^A K_D^B + 2 K_D^B S k_1^A k_{ET}^2 K_D^A + K_D^B S^2 k_3^2 k_{ET} + K_D^B S^2 k_3 k_{ET} k_{-1}^B + K_D^B S^2 k_3^2 k_1^A + K_D^B S k_3 k_{ET} k_{-1}^A K_D^A + S^2 k_3 k_{ET}^2 K_D^B + k_{ET}^2 S^2 k_1^A K_D^B S^2 k_3^2 k_1^A + K_D^B S k_3 k_{ET} k_{-1}^A K_D^A + S^2 k_3 k_{ET}^2 K_D^A + k_1^A k_{ET}^2 (K_D^A)^2 K_D^B + S^3 k_3 k_{ET}^2 + S^3 k_3^2 k_{ET} + S^3 k_3^2 k_1^B + k_{ET}^2 S^3 k_1^B + k_{ET} k_3 S^3 k_1^B + S^2 k_3 k_1^B k_{ET} K_D^A + S^2 k_3 k_{ET} k_{-1}^A K_D^A + k_{ET}^2 S k_1^B (K_D^A)^2 + S^2 k_3 k_{ET}^2 K_D^A + S^3 k_3 k_1^B k_{-1}^B k_{ET} + 2 k_1^B k_1^B k_{ET}^2 S^2 K_D^A)$$