Supporting Information

Sulfido and cysteine ligation changes at the molybdenum cofactor during substrate conversion by formate dehydrogenase (FDH) from *Rhodobacter capsulatus*

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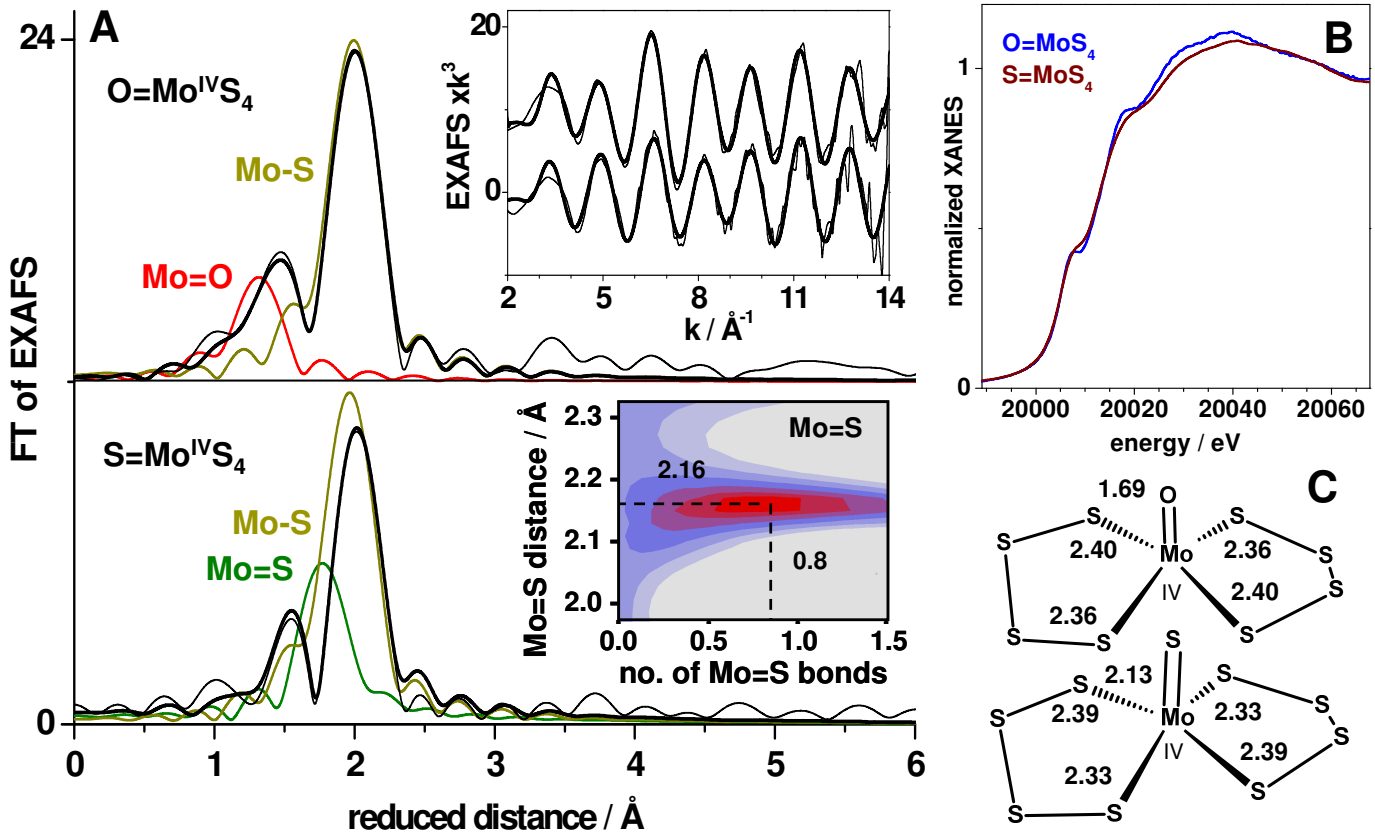

Figure S1: Mo-XAS analysis of two synthetic model complexes. The complexes contained Mo(IV) and carried either a sulfido (bottom) or an oxo (top) ligand. Spectra were obtained for powder samples of the complexes. (A) FTs of EXAFS spectra in the upper inset (thin black lines, experimental data; thick black lines, simulations with parameters in Table S1; coloured lines show the contributions of Mo=O/S and Mo-S ligands to the EXAFS. Lower inset: R_F contour plot for N(Mo=S) and R(Mo=S) variation in the EXAFS fitting for the sulfido complex (red colours, highest fit qualities). (B) XANES spectra revealing similar pre-edge amplitudes (at ~200012 eV) for Mo=S and Mo=O binding. (C) Crystal structures of the complexes[1] in schematic representation with indicated bond lengths (in Å).

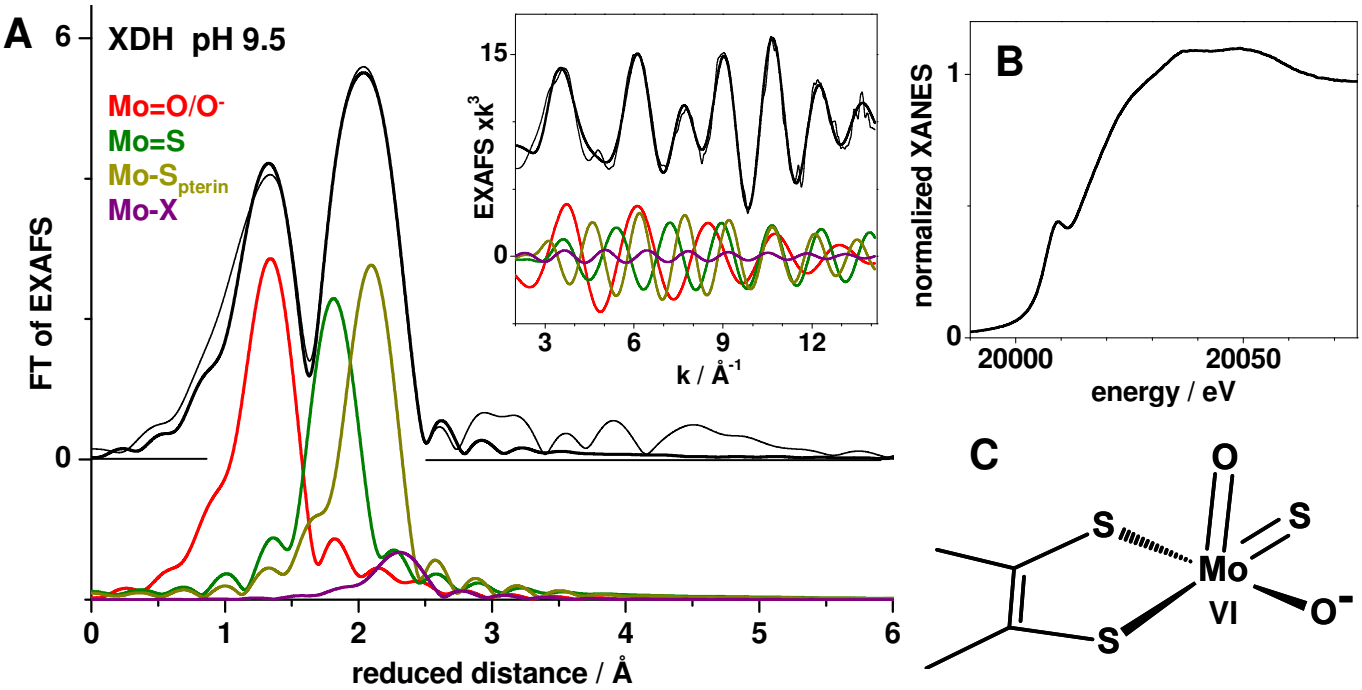

Figure S2: The Mo=S bond in xanthine dehydrogenase. (A) FT of EXAFS spectrum in the inset for XDH protein at pH 9.5 (thin line, experimental data; thick line, simulation with parameters in Table S1) and individual EXAFS contributions of the molybdenum ligands (coloured lines). (B) XANES spectrum. (C) Schematic drawing of the Mo site structure in XDH showing one pyranopterin ligand, one sulfido bond, and two oxygen ligands.[2]

Table S1: EXAFS fit results for reference samples.[a]

	Mo=O,-O	Mo=S	Mo-S	Mo···X	
sample	N [per Mo] / R [Å] / $2\sigma^2$ x10^3 [Å^2]				R_F [%]
(O=)MoS_4	0.85 / 1.68 / 2	0.15 / 2.16 / 2	4.08 / 2.38 / 6		9.1
(S=)MoS_4	0.14 / 1.67 / 2	0.86 / 2.16 / 2	3.90 / 2.37 / 6		10.2
XDH pH 9.5	2.32 / 1.72 / 11		1.59 / 2.47 / 1		42.0
	2.09 / 1.73 / 8	1.33 / 2.18 / 8	2.28 / 2.48 / 6		18.2
	2.13 / 172 / 8	1.36 / 2.19 / 8	2.20 / 2.48 / 6	0.57 / 2.88 / 6	10.2
	0.92 / 1.69 / 2 1.08 / 1.77 / 2	0.97 / 2.18 / 2	2.02 / 2.48 / 5	0.51 / 2.88 / 5	8.7
	0.95 / 1.69 / 2 1.05 / 1.77 / 2	1.03 / 2.18 / 2	1 / 2.44 / 2 1 / 2.52 / 2	0.34 / 2.88 / 2	7.8

[a]For fit restraints see the legend of Tables 3 and S3, for fit curves see Figs. S1 and S2. Data for xanthine dehydrogenase (XDH) show the gradual refinement of the fit approach. Small N-values of Mo=O bonds for (S=)MoS_4 or Mo=S bonds for (O=)MoS_4 are insignificant and such ligands therefore were likely absent.

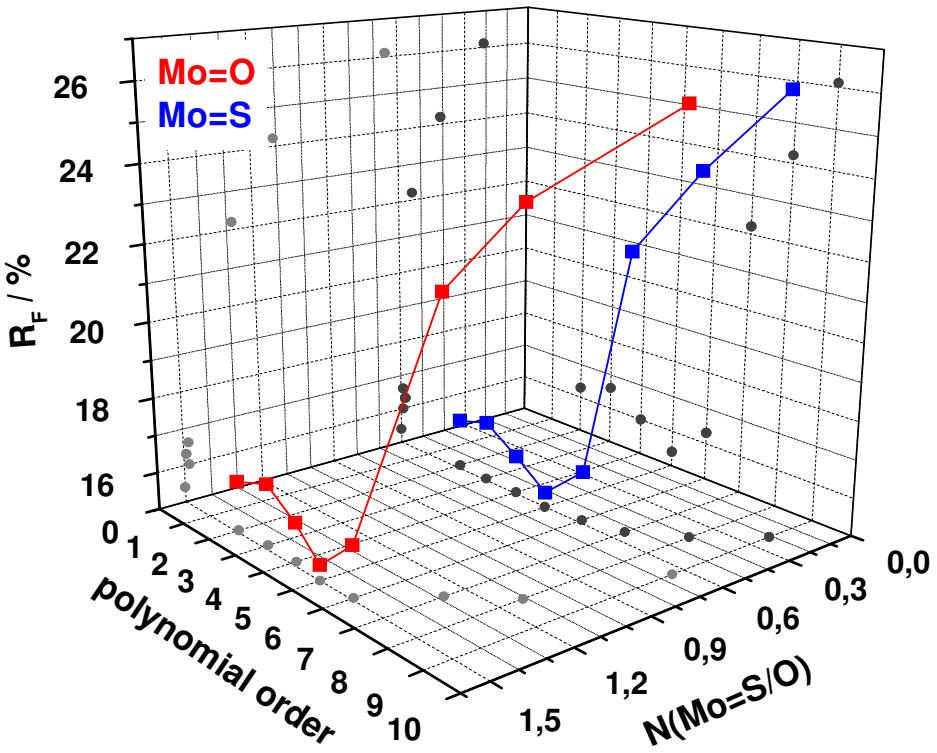

Figure S3: Mo=S vs. Mo=O coordination in *Rc*FDH. Shown are changes in the EXAFS fit results of *Rc*FDH$_{WT}^{ox}$ for varying background removal in the XAS data processing. Data refer to the spectrum in Fig.3 (middle). An increasing order of the polynomial used for background removal in the EXAFS data processing procedure[3] lead to the parallel decrease of Mo=O and Mo=S coordination numbers (*N*) derived from fit analysis of the resulting EXAFS spectra (not shown) and to a low fit quality (high R_F value) (for inclusion of 4 Mo-S(MGD) bonds in the fits). Overestimation of *N*(Mo=O) in the *Rc*FDH samples therefore was unlikely.

Table S2: Estimation of cofactor species distribution in *Rc*FDH$_{WT}^{ox}$ samples.[a]

*Rc*FDH$_{WT}^{ox}$	Mo-site population [%]			N [per Mo]
pH	MoS_4O	MoS_2O_3	MoS_6	Mo=O/-O$^-$ calculated / experimental
7.0	70	15	15	1.15 / 1.20[b]
8.0	30	35	35	1.35 / 1.35[b]
9.0	0	50	50	1.50 / 1.60[b]

[a]See Fig. 3 for EXAFS spectra, [b]data refer to fits with $N = 6$ in the Mo 1st-sphere (Table 3).

Table S3: Additional EXAFS simulation parameters for *Rc*FDH proteins.[a]

		Mo=O,-O	Mo=S	Mo-S	Mo···X		
*Rc*FDH	fit	N [per Mo] / R [Å] / $2\sigma^2$ x10^3 [Å^2]				R_F [%]	BVS
WT							
(ox) pH 7	1	0.61 / 1.67 / 2 0.59 / 1.77 / 2	0.14 / 2.16 / 3	4 / 2.37 / 28 0.28 / 2.81 / 3	1.79 / 2.85 / 28	6.4	5.70
	2[b]	0.84 / 1.68 / 2 0.74 / 1.78 / 2	0.21 / 2.15 / 3	4 / 2.36 / 27 0.21 / 2.81 / 3	1.79 / 2.85 / 27	10.2	6.39
(ox) pH 8	3	0.81 / 1.68 / 2 0.50 / 1.78 / 2	0.40 / 2.16 / 3	4 / 2.37 / 17 0.35 / 2.68 / 3	1.34 / 2.86 / 17	4.3	6.35
	4[b]	0.83 / 1.68 / 2 0.44 / 1.78 / 2	0.36 / 2.16 / 3	4 / 2.36 / 17 0.36 / 2.67 / 3	1.64 / 2.85 / 17	7.5	6.37
(ox) pH 9	5	1.21 / 1.70 / 2 0.47 / 1.81 / 2	0.58 / 2.17 / 3	4 / 2.37 / 13 0.72 / 2.63 / 3	1.26 / 2.84 / 13	5.6	7.27
	6[b]	0.83 / 1.70 / 2 0.35 / 1.80 / 2	0.41 / 2.17 / 3	4 / 2.38 / 14 0.41 / 2.64 / 3	1.59 / 2.86 / 14	12.4	6.14
(red) pH 9	7	0.89 / 1.72 / 2 0.21 / 1.88 / 2	0.49 / 2.23 / 3	4 / 2.41 / 12 0.69 / 2.63 / 3	1.23 / 2.84 / 12	5.3	5.79
	8[b]	0.86 / 1.71 / 2 0.20 / 1.86 / 2	0.45 / 2.20 / 3	4 / 2.41 / 13 0.45 / 2.63 / 3	1.55 / 2.84 / 13	8.6	5.61
(formate) pH 7	9	0.80 / 1.69 / 2 0.71 / 1.79 / 2	0.12 / 2.15 / 3	4 / 2.36 / 24 0.39 / 2.65 / 3	1.41 / 2.84 / 24	4.5	6.22
	10[b]	0.76 / 1.68 / 2 0.82 / 1.79 / 2	0.21 / 2.15 / 1	4 / 2.37 / 21 0.21 / 2.67 / 3	1.79 / 2.84 / 21	9.2	6.30
(formate) pH 9	11	1.13 / 1.73 / 2 0.75 / 2.23 / 2	0.53 / 2.19 / 3	4 / 2.37 / 16 0.43 / 2.89 / 3	0.77 / 3.01 / 16	5.0	6.29
	12[b]	1.04 / 1.73 / 2 0.48 / 2.23 / 2	0.48 / 2.20 / 3	4 / 2.38 / 14 0.52 / 2.88 / 3	1.48 / 3.03 / 14	9.4	5.73
ΔFdsC							
(ox) pH 7	13	0.46 / 1.68 / 2 0.51 / 1.76 / 2	0.15 / 2.17 / 3	4.41 / 2.42 / 14 0.36 / 2.73 / 3	0.82 / 2.88 / 14	8.6	5.17
	14[b]	0.56 / 1.68 / 2 0.44 / 1.77 / 2	-	4 / 2.41 / 15 1 / 2.56 / 3	1[*] / 2.81 / 15	12.4	5.23
(ox) pH 9	15	0.58 / 1.69 / 2 0.36 / 1.79 / 2	0.12 / 2.17 / 3	4.67 / 2.43 / 12 0.31 / 2.73 / 3	0.79 / 2.80 / 12	6.6	5.24
	16[b]	0.69 / 1.70 / 2 0.31 / 1.80 / 2	-	4 / 2.43 / 17 1 / 2.43 / 3	1[*] / 2.86 / 16	11.0	5.39
C386S							
(ox) pH 7	17	0.63 / 1.68 / 2 0.49 / 1.78 / 2 0.57 / 2.41 / 3	0.31 / 2.14 / 3	4 / 2.33 / 23	1 / 2.82 / 23	9.3	6.30
H387M							
(ox) pH 7	18	0.86 / 1.69 / 2 0.40 / 1.80 / 2	0.38 / 2.16 / 3	4 / 2.38 / 16 0.42 / 2.67 / 3	1.21 / 2.87 / 17	7.5	6.15
	19[b]	0.87 / 1.68 / 2 0.41 / 1.80 / 2	0.35 / 2.16 / 3	4 / 2.37 / 17 0.35 / 2.68 / 3	1.65 / 2.88 / 18	9.5	6.14
(ox) pH 9	20	1.07 / 1.74 / 2 0.64 / 2.21 / 2	0.07 / 2.17 / 3	4 / 2.37 / 22 0.32 / 2.65 / 3	1.53 / 2.83 / 22	7.3	5.61
	21[b]	0.98 / 1.74 / 2 0.75 / 2.19 / 2	0.13 / 2.17 / 3	4 / 2.37 / 23 0.13 / 2.63 / 3	1.87 / 2.84 / 23	9.0	5.56

[a]Fit restraints: $2\sigma^2$ values for Mo=O,-O, Mo=S, and the second Mo-S bond were fixed. [b]The summed *N*-value of distances <2.7 Å was set to 6. For further details see Table 3.

Supporting references

(1) Draganjac, M.; Simhon, E.; Chan, L. T.; Kanatzidis, M.; Baenziger, N. C.; Coucouvanis, D. *Inorg Chem* **1982**, *21*, 3321-3332.
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(3) Dau, H.; Liebisch, P.; Haumann, M. *Anal Bioanal Chem* **2003**, *376*, 562-583.