Supporting Information

Anion binding and oxidative modification at the molybdenum cofactor of formate dehydrogenase from *Rhodobacter capsulatus* studied by X-ray absorption spectroscopy

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Table S1: Preparation procedures of *Rc*FDH proteins.[a]

aerobic (O_2) *Rc*FDH purification from *E. coli* with:	azide (N_3^-)				cyanate (OCN^-) 10 mM
	0.5 mM		10 mM		
anion removal:	aerobic (O_2) desalting[c]	anaerobic desalting		-	-
anion addition:	-		cyanide (CN^-) 10 mM[b]	-	-
preparation type:	1	2	3	4	5
treatment:[d]	as isolated, NADH, formate (HCO_2^-)				
concentration:	removal of excess anions and redox agents				

[a]The sodium or potassium salts of the anions were added to the buffers. [b]KCN was added to samples containing ~100 µM *Rc*FDH. [c]Samples were made anaerobic after desalting prior to addition of NADH or formate. [d]Proteins of types 1-5 (~100 µM *Rc*FDH) were concentrated to ~1 mM Mo content in as-purified form (as isolated), or after treatment by addition of 10 mM NADH or 10 mM formate. Periods of exposure of proteins to aerobic conditions are marked by (O_2).

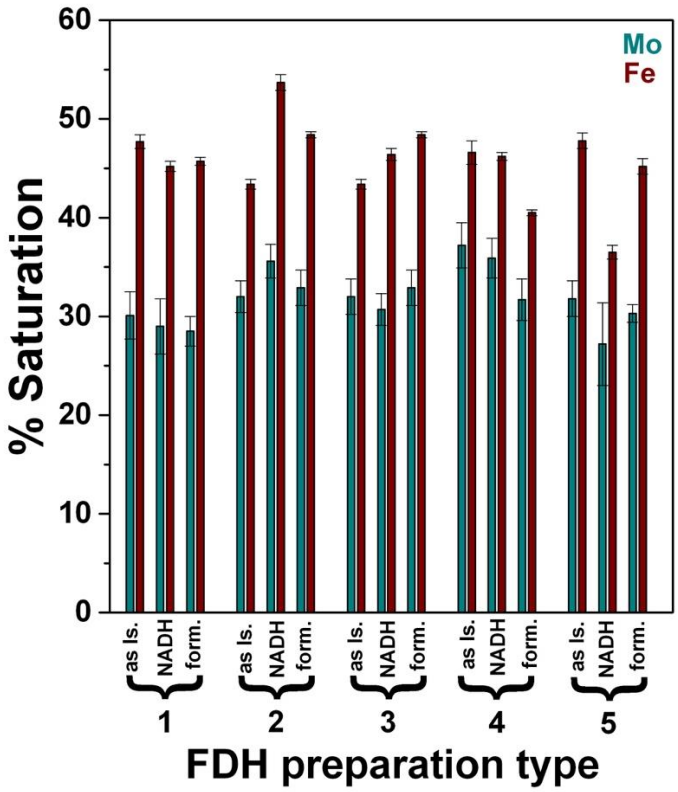

Figure S1: Molybdenum and iron saturations for *R. capsulatus* FDH samples from ICP-OES. Sample type annotations are as in Table S1 (as is., as isolated; NADH, NADH reduced; form., formate reduced). Metal contents were determined for aliquots of the FDH protein samples that were taken before the samples were loaded into acrylic-glass sample holders, frozen in liquid nitrogen, and used in the XAS experiments. Values represent the mean of duplicate measurements on samples containing 10-20 µM FDH (error bars show the full variation range), as described elsewhere (Hartmann et al., FEBS J. 280, 6083-6096, 2013).

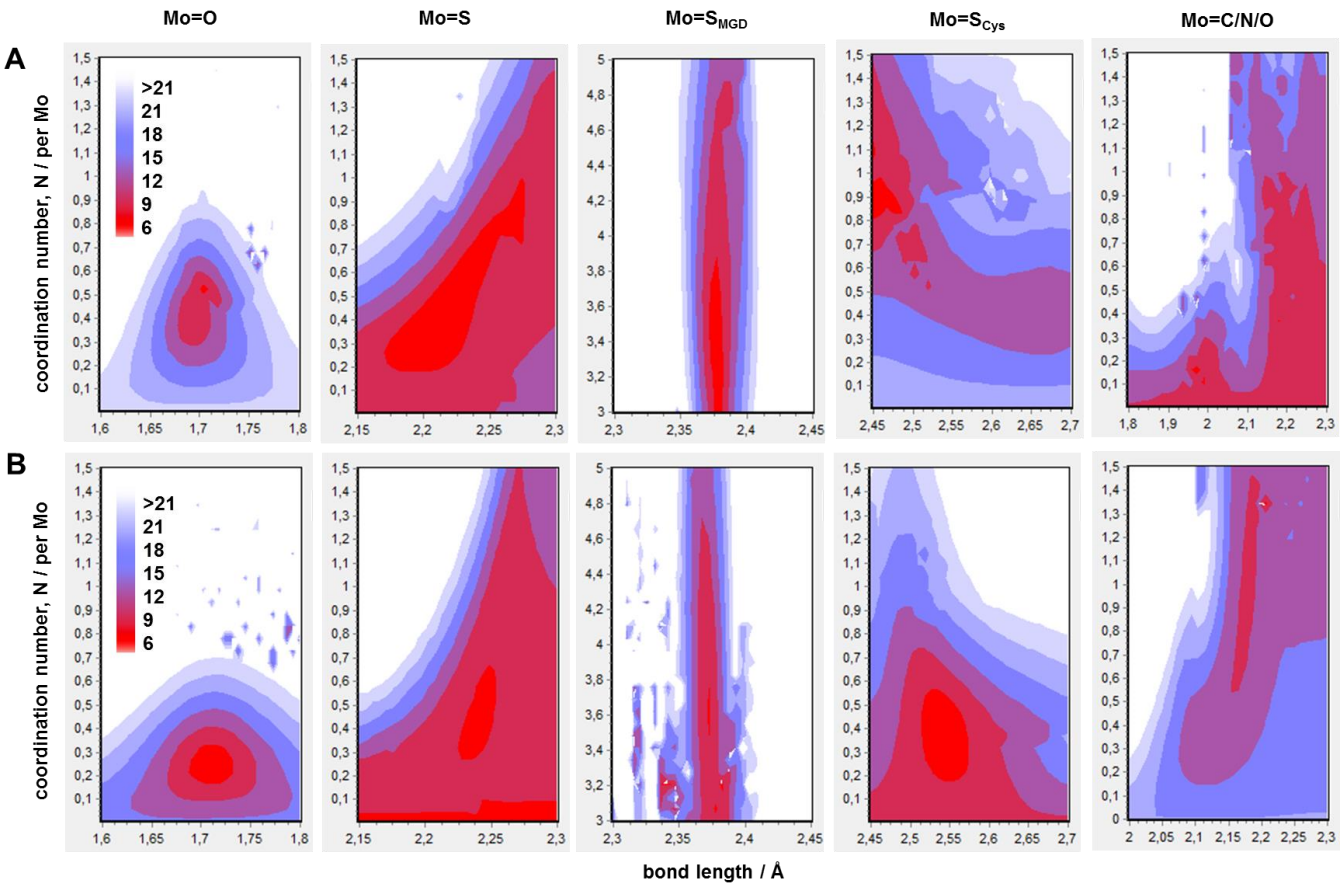

Figure S2: Error contour plots from fits of FDH EXAFS spectra. Data correspond to as-isolated FDH samples in Table S1 of (A) type 2 (0.5 mM azide, anaerobic desalting) and (B) type 4 (10 mM azide). A fit approach with 5 ligand shells was used. The left panels in A and B show the color code of the fit error (R_F in %) in the contour plots as derived from variation of the coordination numbers (N, y-axis) and bond lengths (R, x-axis). From similar calculations for all EXAFS spectra, approximate error margins of the N and R values were estimated as given in the Table 1 caption.

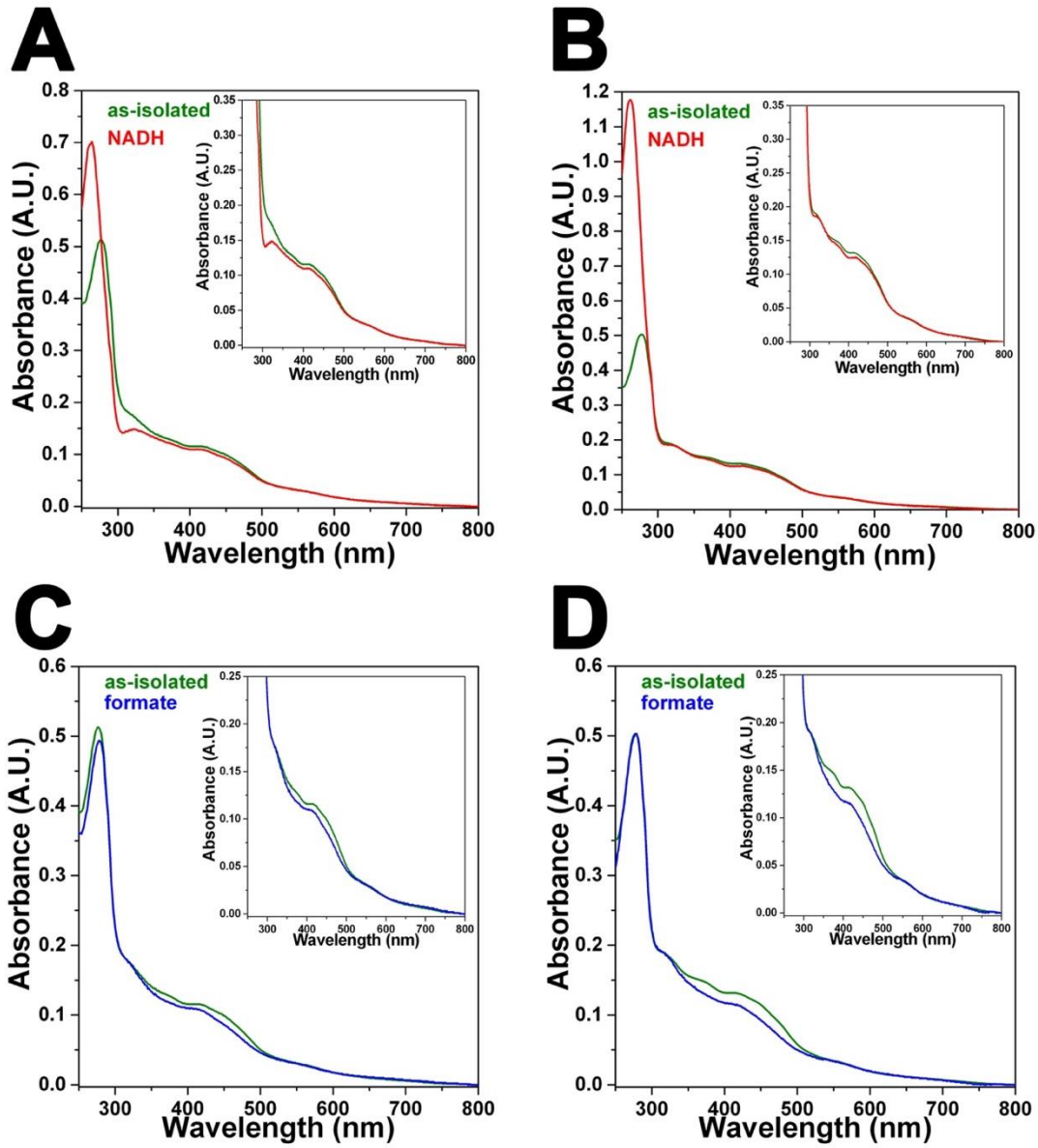

Figure S3: UV-visible spectra of selected FDH sample preparations for XAS. (A) and (C) refer to type-2 samples (0.5 mM azide, anaerobic desalting) handled anaerobically in the absence of azide in the as-isolated and NADH-treated states, while (B) and (D) refer to type-4 samples (10 mM azide) handled aerobically in the presence of azide in the as-isolated and formate-treated states. Spectra correspond to a diluted aliquot of the respective sample that was taken prior to loading into the sample frame for XAS, corresponding to approximately 2.9 µM FDH. As-isolated spectra correspond to respective "as-isolated" XAS samples shown in Figures 3-6 and are depicted relative to formate or NADH treatment samples for comparison. Spectra were measured in 100 mM Tris-HCl (pH 9.0) in the absence (A) or presence (B) of 10 mM NaN_3. Overlaid spectra were normalized to their absorbance at 550 nm, since particularly in the NADH-treated samples the high absorbance at 280 nm is due to the presence of NAD^+. Insets depict a magnification of the spectral features in the 250-800

nm region. In (A) the loss of absorbance at 320 nm reflects loss of the sulfido ligand and/or damage of a Fe-S cluster. The presence of azide depicted in (B) seems to prevent damage of the protein. The decrease in 450 nm absorbance depicted in (C) and (D) upon reduction with formate is attributed to loss of the FMN cofactor. In contrast to (B), azide is not preventing this damage, since a comparable decrease in absorbance is observed in (C) and (D).