Supplementary Material

FTIR studies on sulfide binding to the Ni-Fe site.

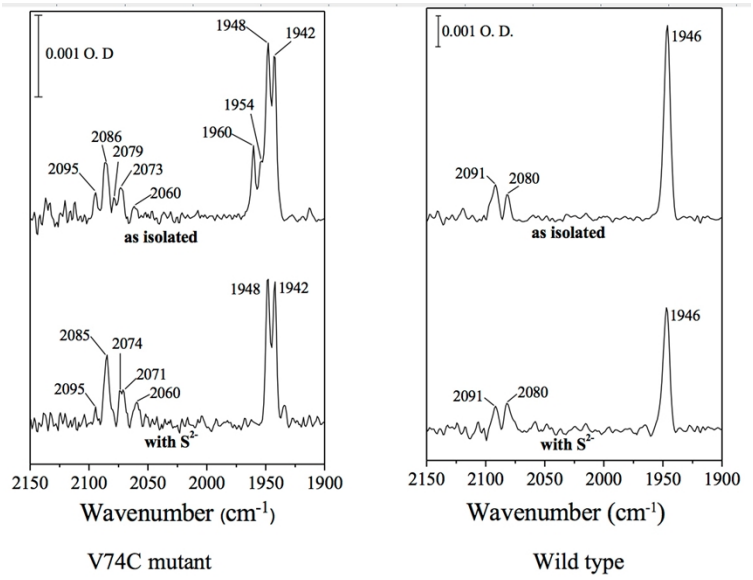

Figure S1. Comparative FTIR spectra of 0.1 mM samples of the as isolated *air-exposed* V74C mutant and the wild type (WT) *D. fructosovorans* [NiFe]-hydrogenase before (top) and after full activation and subsequent reoxidation (bottom). Both samples were first fully activated for 4 hours at room temperature under a hydrogen atmosphere. Next, hydrogen was replaced by argon and Na_2S was added anaerobically to a final concentration of 4 mM. The enzyme samples were then reoxidized by air giving the spectra labelled "with S^{2-}" (bottom). For the mutant the bands at 1948, 2085 and 2095 cm^{-1} are assigned to the unready Ni-A state. The bands at 1942, 2060 and ≈2073 cm^{-1} are not observed after reoxidation in the absence of Na_2S (ref. 18) and therefore indicate an S-bound state. The origin of the CO bands at 1954 and 1960 cm^{-1} is unclear. A similar treatment of the WT enzyme produced only bands at 1946, 2080 and 2091 cm^{-1}, which are known to correspond to the Ni-B state. After thawing the anaerobically purified WT sample under air, also only the Ni-B state was detectable (top of right panel). This indicates that this sample was sufficiently reduced to prevent the formation of unready states. In addition, this experiment confirmed that no unready states were formed during purification. It is not clear why in the presence of sulfide so much Ni-B state was formed after activation and reoxidation of the WT enzyme.