Supporting Information

Infrared Characterization of the Bidirectional Oxygen-Sensitive [Nife]-Hydrogenase from *E. Coli*

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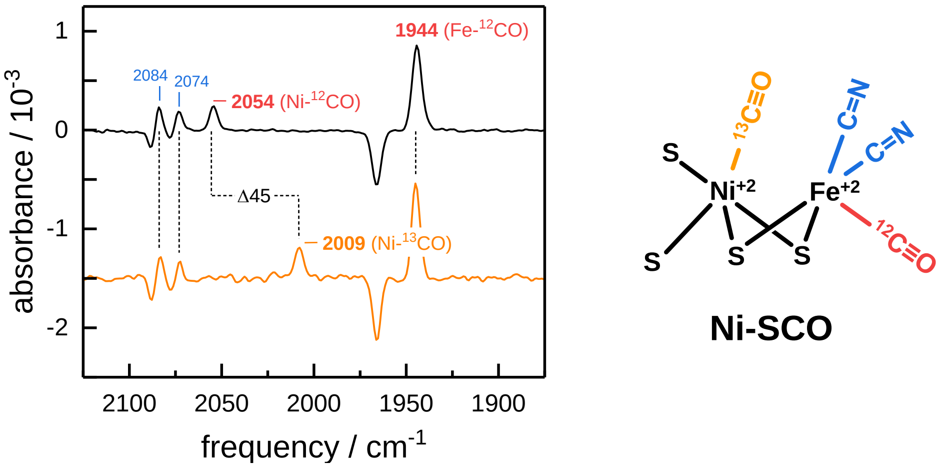

Figure S1. ^{13}CO isotope editing. The black difference spectrum shows the enrichment of Ni-SCO over Ni-C and Ni-SI upon ^{12}CO binding the nickel ion (see main script). In the presence of ^{13}CO, the Ni-CO signal shifts from 2054 to 2009 cm^{-1} (orange difference spectrum). The observed shift of 45 cm^{-1} to lower frequencies is explained by the difference in reduced mass $\mu = \frac{m1m2}{m1+m2}$ between ^{12}CO and ^{13}CO. The Fe(CN)$_2$CO signature remains unaffected by the isotope editing, which proves the presence of an individual CO ligand at the nickel site.

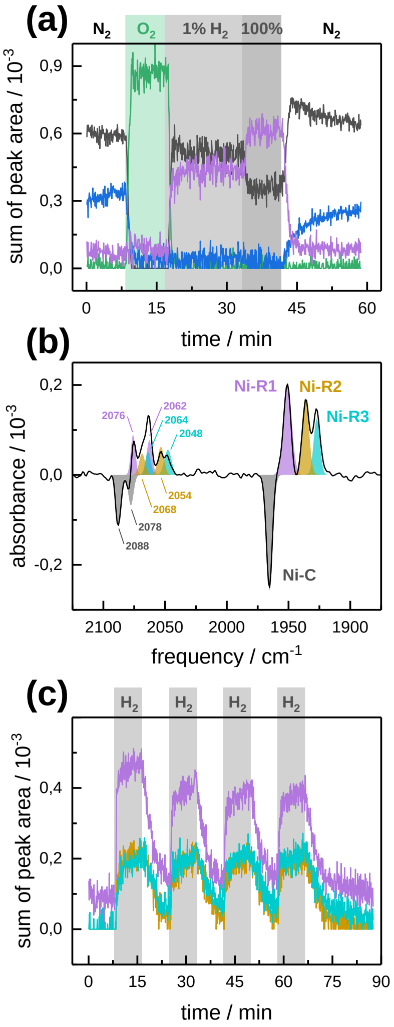

Figure S2. Further H_2 titrations and Ni-R band assignment. **(a)** In order to assign the Fe(CN)$_2$CO signature of the Ni-R states the following procedure was attempted. First, the sample was brought in contact with 1% O_2 to homogenize the film and enrich Ni-B over the as-isolated mixture of Ni-C and Ni-SI (see Fig. 6 in the main script). Second, O_2 was replaced by 1% H_2 to reductively activate the enzyme. The reduced species Ni-C and Ni-R were found to dominate the spectrum with no increase of the oxidized Ni-SI in the presence of H_2. However, the H_2 – O_2 difference spectrum still included the negative bands of Ni-B. Although the film was completely reactivated with 1% H_2, a further increase of Ni-R over Ni-C was observed by switching the gas stream to 100% H_2. **(b)** "100% H_2 – 1% H_2" difference spectrum that depicts the increase of Ni-R1 – R3 over Ni-C. The Fe(CN)$_2$CO signature of Ni-C was identified before (see main script). Based on the singular peaks at 2076, 2054, and 2048 cm^{-1} and the observation that both CO and CN$^-$ bands shift in the same direction, a unique fit was achieved for the Fe(CN)$_2$CO assignment of Ni-R1 – R3 (see Tab. 1 in the main script). **(c)** This data was used to describe the concomitant increase and decrease of R-states, e.g., depending on the presence or absence of H_2 from the carrier gas.

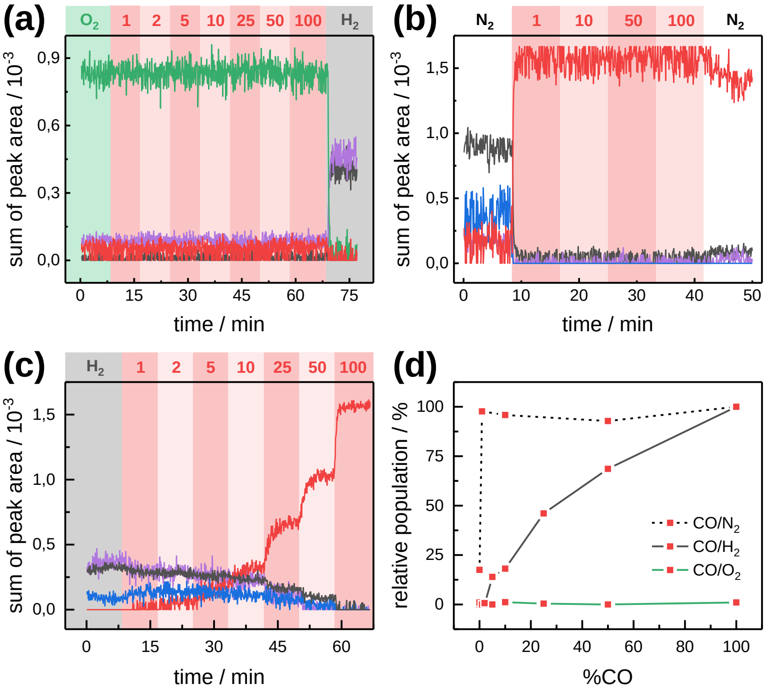

Figure S3. Further CO titrations. **(a)** In the presence of O_2 carrier gas, no CO inhibition was observed even when the gas stream was switched to 100% CO. The reaction with O_2 very efficiently removes Ni-SI from the equilibrium of species. Therefore, CO cannot bind to the [NiFe] active site. **(b)** With N_2 as carrier gas, 1% CO in the gas stream completely converts the film into Ni-SCO. The mixture of Ni-C and Ni-SI (characteristic for HYD-2 under inert gas) facilitates an immediate conversion into Ni-SCO in the presence of CO. **(c)** With H_2 as carrier gas, an apparent decrease in the CO affinity is observed (see main script). **(d)** When the relative population of Ni-SCO is plotted against CO ambient partial pressure, the differences in CO affinity as a function of carrier gas can be visualized in a single graph.