# Probing the origin of the metabolic precursor of the CO ligand in the catalytic center of [NiFe]-hydrogenase

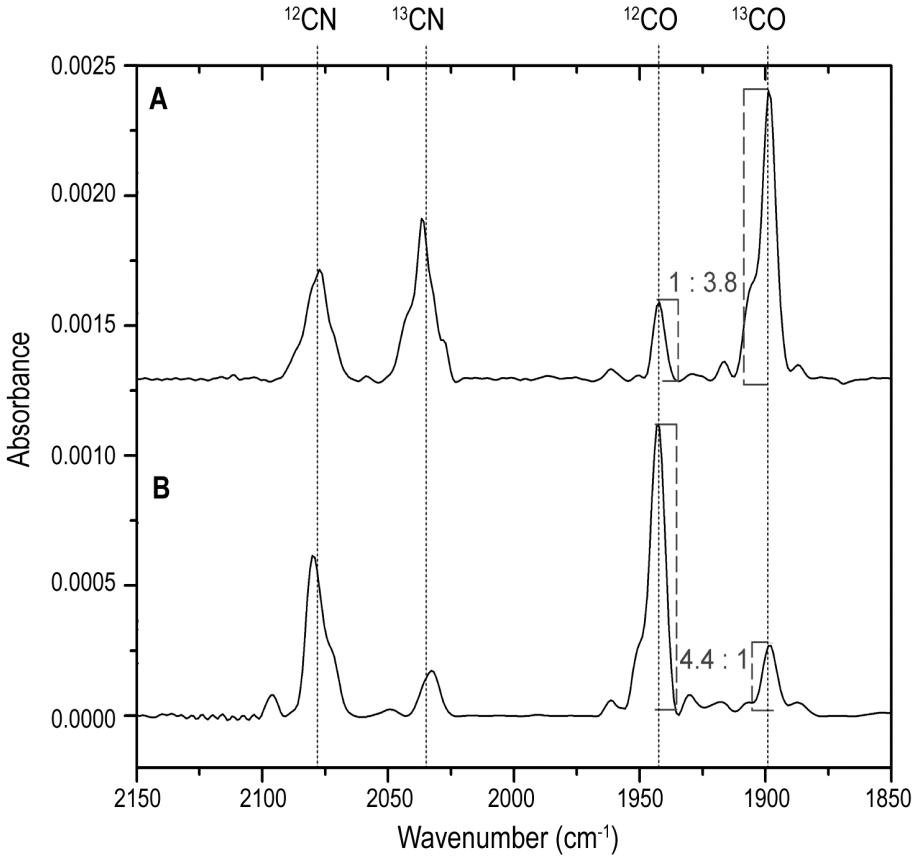
**Ingmar Bürstel[1], Philipp Hummel[2], Elisabeth Siebert[2], Nattawadee Wisitruangsakul[2], Ingo Zebger[2], Bärbel Friedrich[1], and Oliver Lenz[1]**

[1]Humboldt-Universität zu Berlin, Institut für Biologie/Mikrobiologie, Chausseestraße 117,10115 Berlin, Germany.
[2]Technische Universität Berlin, Max-Volmer-Laboratorium, Straße des 17. Juni 135, 10623 Berlin, Germany.

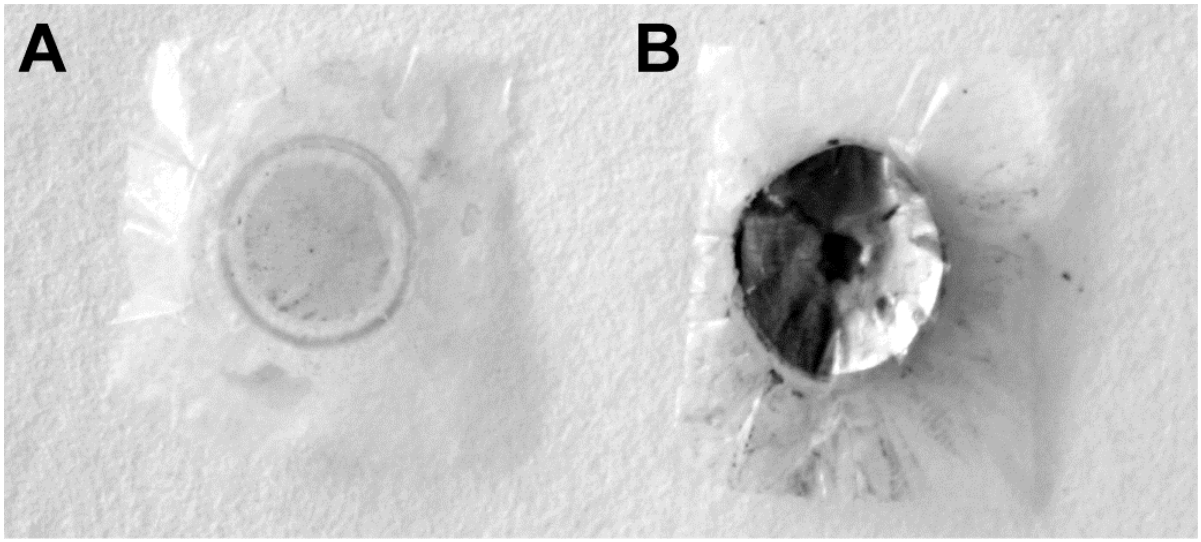
**Supplemental Data**

**Table of content**






SUPPLEMENTAL FIGURE S1. **FTIR spectroscopic analysis of the regulatory [NiFe]-hydrogenase purified from cells grown heterotrophically on fructose and differentially labeled glycerol.** The following substrates were used: *A*, $^{12}C_6$-fructose and 1,3-$^{13}C_2$-glycerol; *B*, $^{12}C_6$-fructose and 2-$^{13}$C-glycerol. Hatched lines indicate the ν(CN) and ν(CO) vibrational modes. Bands at wavenumbers 2080 $cm^{-1}$ and 2072 $cm^{-1}$ are attributed to the $^{12}CN^-$ ligands and the absorption at 1943 $cm^{-1}$ to the $^{12}$CO ligand. Incorporation of the $^{13}$C atom leads to specific band shifts to 2038 $cm^{-1}$ and 2027 $cm^{-1}$ of the $CN^-$ ligands and to 1899 $cm^{-1}$ of the CO ligand.




SUPPLEMENTAL FIGURE S2. **CO-mediated reduction of $PdCl_2$ resulting in the formation of metallic Pd.** Palladium(II)chloride-filled capsules sealed with a gas-permeable membrane were deposited in *R. eutropha* cell cultures growing heterotrophically (*A*) in minimal medium with fructose-glycerol, or lithoautotrophically (*B*) on $H_2$ and $CO_2$. The capsules were removed after a cultivation time of 35 h and the membrane was analyzed for the precipitation of metallic palladium.