

# DFT investigation of the Ni-A inactive state of the [NiFe]-hydrogenases: inactivation mechanism under aerobic conditions



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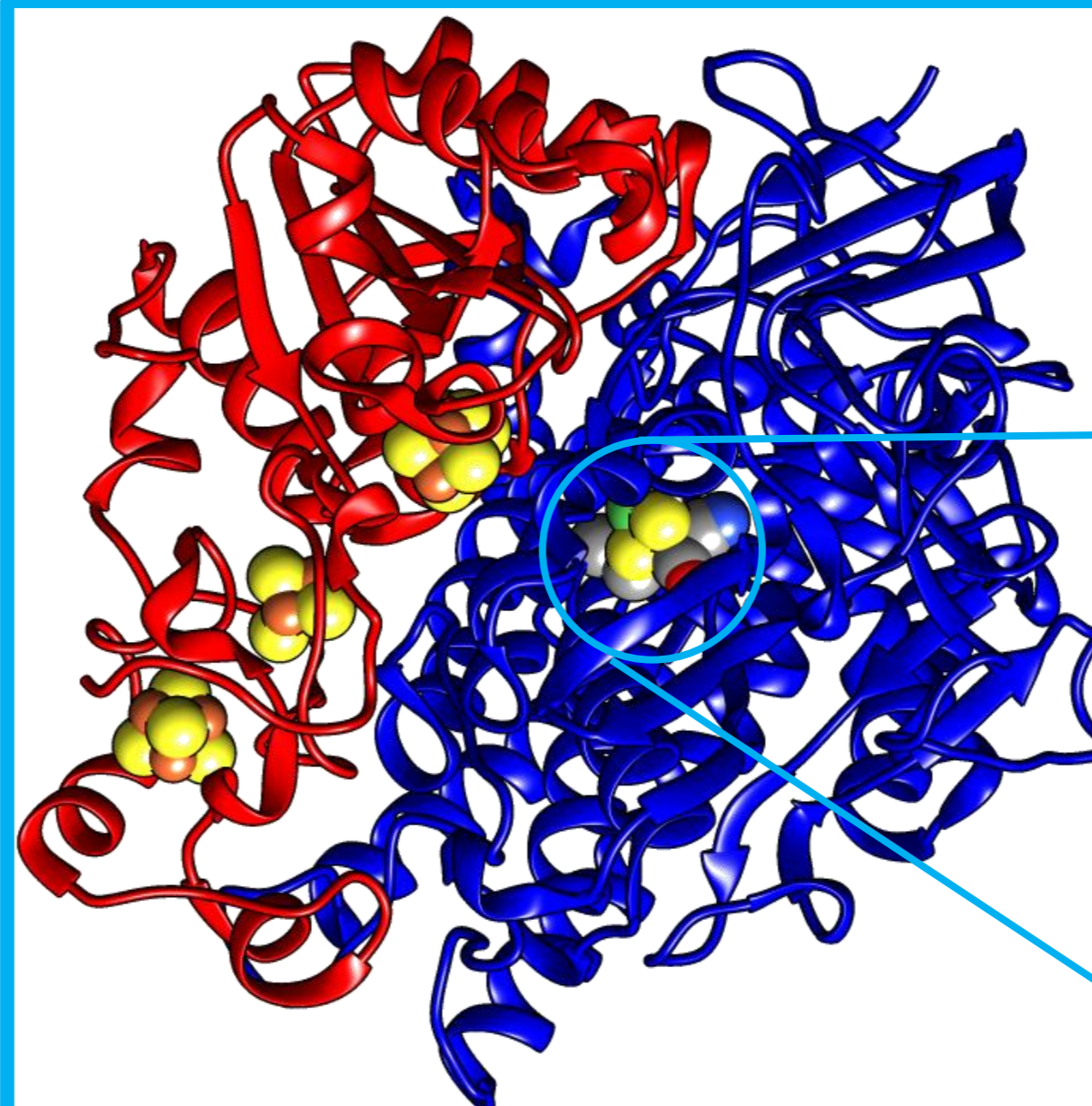
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**Introduction:** Dihydrogen is considered as a future non polluting energy vector. In this context Hydrogenase enzymes are of fundamental interest for their capability to catalyse the reversible interconversion of protons and reducing equivalents into molecular hydrogen. While the [FeFe]-H<sub>2</sub>ases are irreversibly inactivated by O<sub>2</sub>, the oxidized [NiFe]-hydrogenases can be reactivated by one-electron reduction and protonation. This property, in addition to the high catalytic efficiency and the absence of expensive metals in their active site makes the [NiFe]-H<sub>2</sub>ases a very promising target for reverse engineering studies aimed at the development of bioinspired catalysts.

**Aim of the work:** To better characterize the inactive oxidized Ni-A state of this enzyme, quantum mechanics calculations have been carried out in the framework of the Density Functional Theory (DFT) on a very large model of the active site. Mechanisms of oxidation of active forms of the enzyme to the Ni-A and Ni-B states were investigated under aerobic conditions.

## Methods:

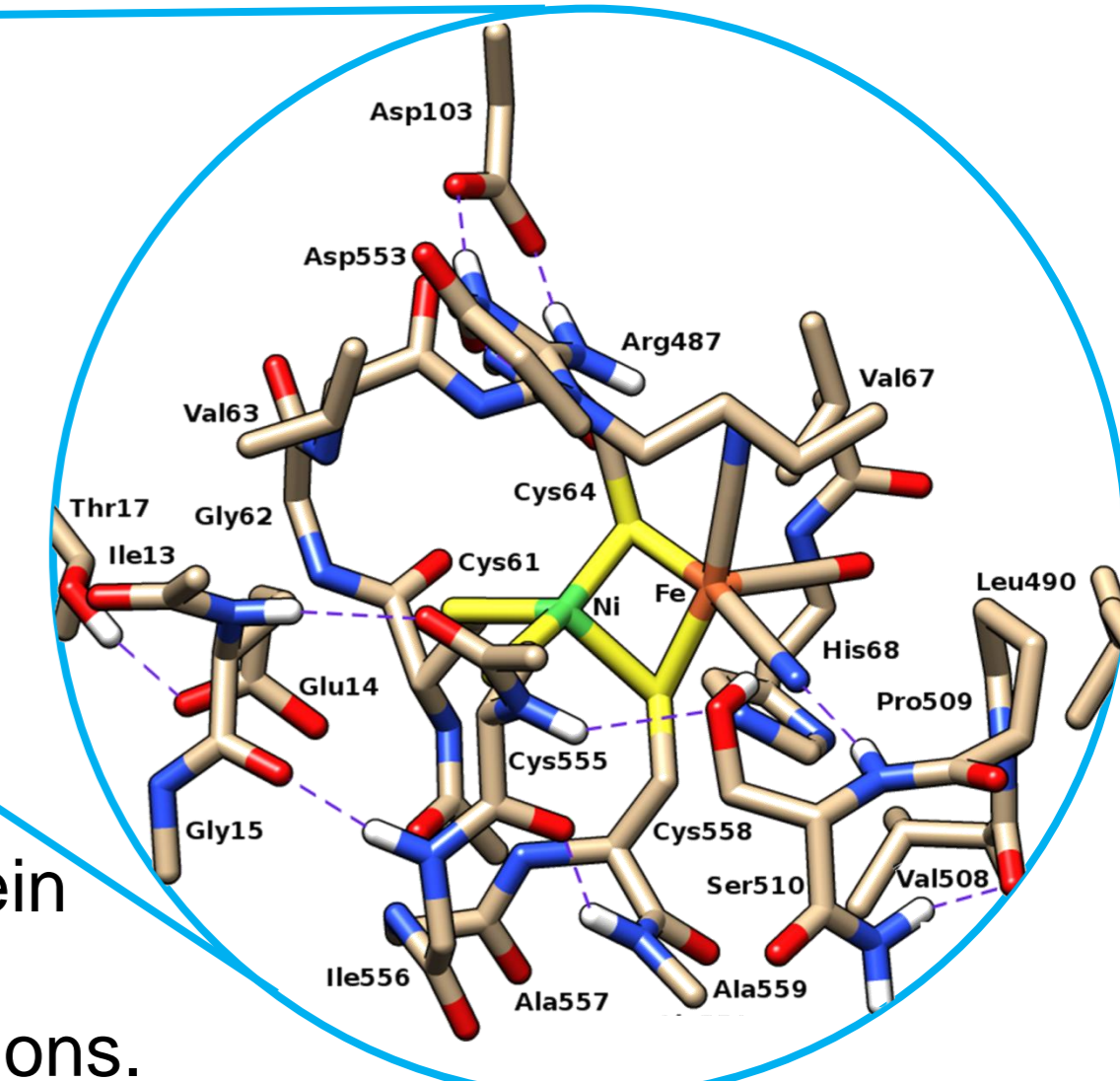
DFT model (**290 atoms**) obtained from the X-ray structure of the active site of *Allochromatium Vinosum* (pdb code **3MYR**)



BP86/def-TZVP-SVP

Continuum dielectric with  $\epsilon=4$  for the protein environment (COSMO)

Terminal atoms restrained to the X-ray positions.



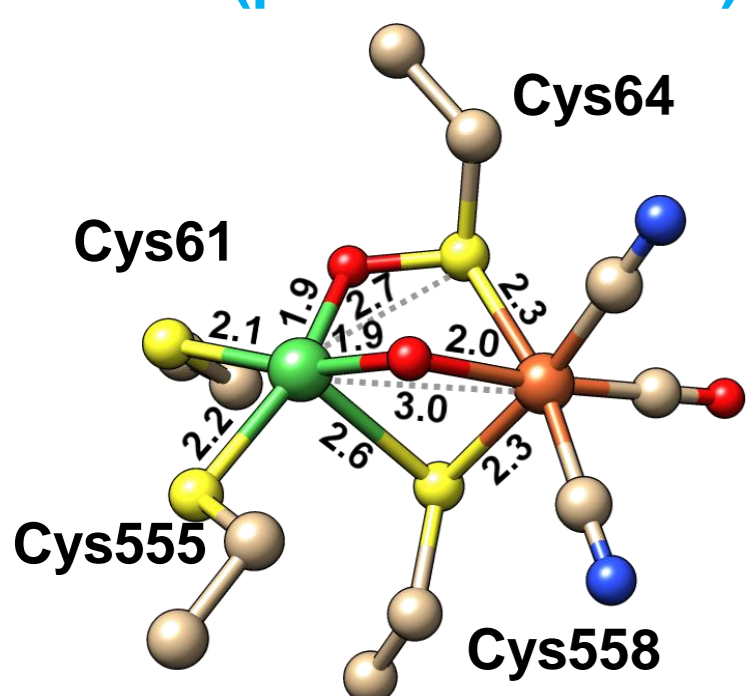
## Ni-A state:

The Ni-A state, described as Ni<sup>III</sup>Fe<sup>II</sup> with an oxygenic bridging ligand, is an **EPR active** oxidized state of the [NiFe]-hydrogenases. Based on crystallographic analyses, different O<sub>2</sub>-reduction products have been proposed to bind to the Ni-A state but its exact nature is still not clear.

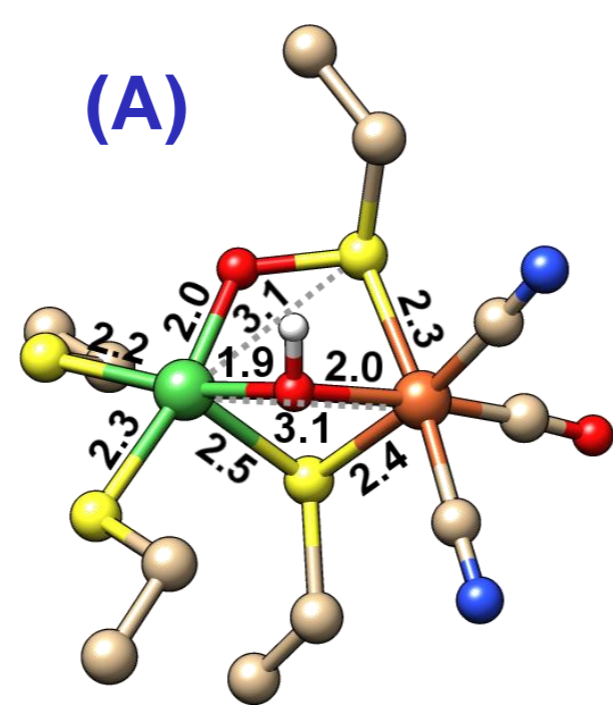
A recent crystallographic structure shows a bridging monoatomic oxygen species and the Cys64 oxidized to its sulfenated form<sup>[1]</sup>

X-ray structure Ni-A state (pdb code 4UPE)

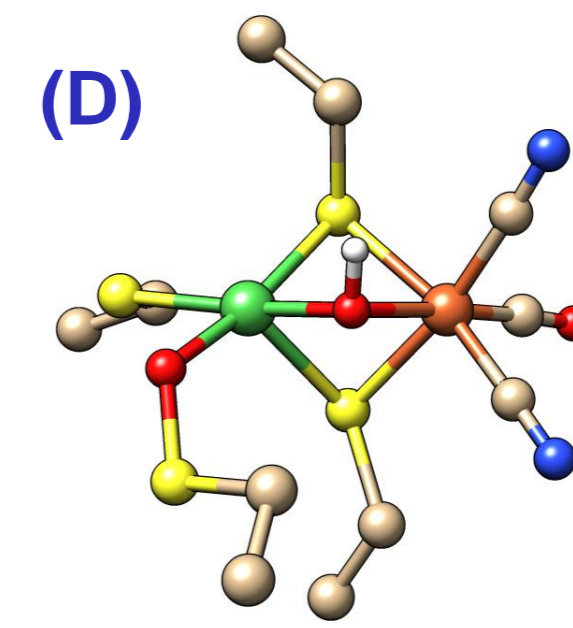
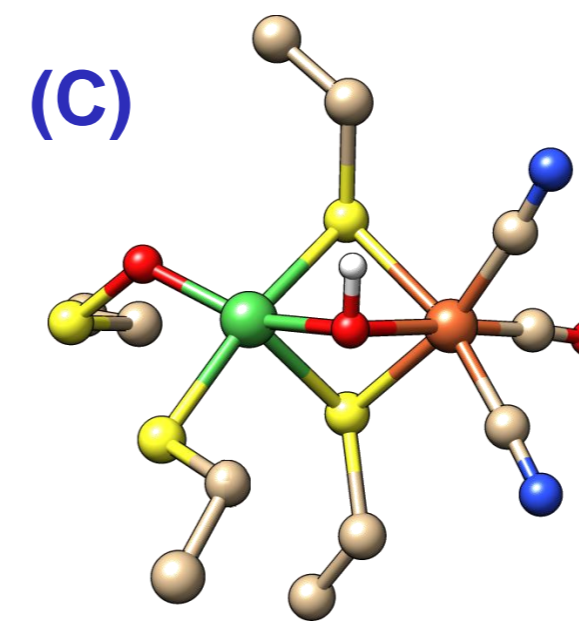
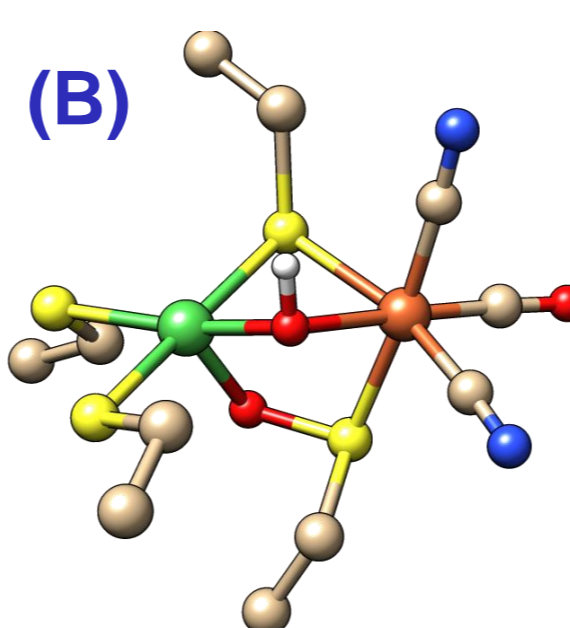
DFT model



X-ray structure is well reproduced by calculations

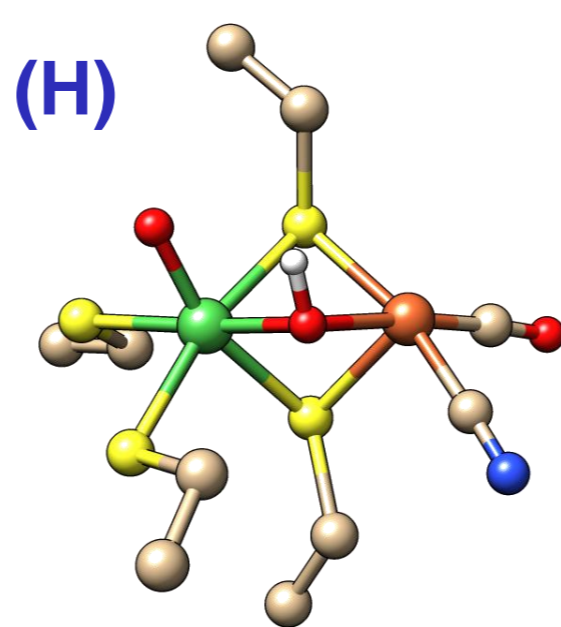
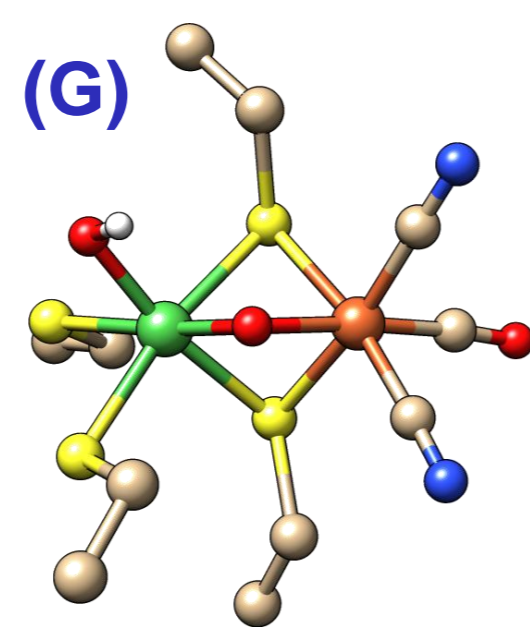
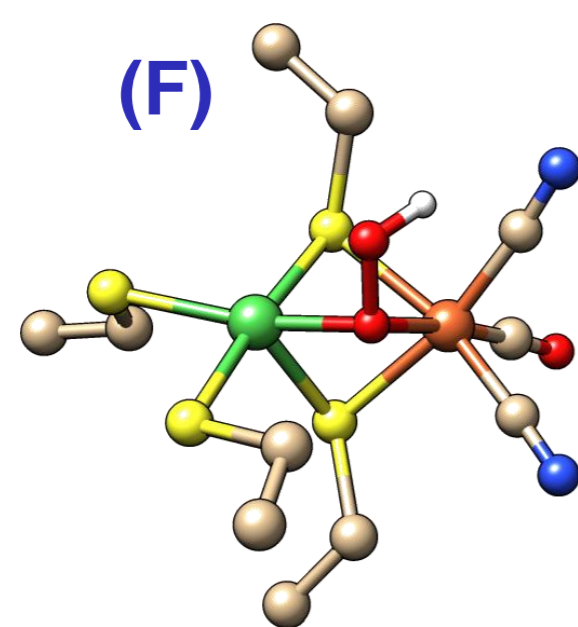
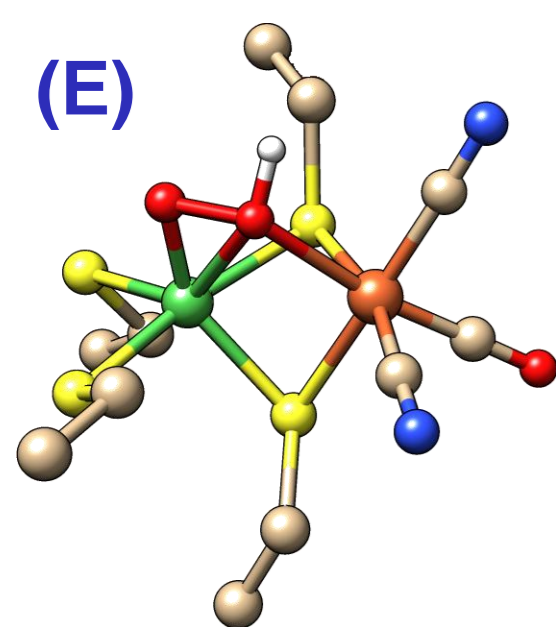


In agreement with the X-ray structure, sulfenated forms of Cys61, Cys555 and Cys558 are energetically disfavoured.



	$E_r$ (kcal/mol)
(A)	0.0
(B)	+11.1
(C)	+11.1
(D)	+8.83

Ni-A structures proposed by previous crystallographic and DFT studies, were also investigated.



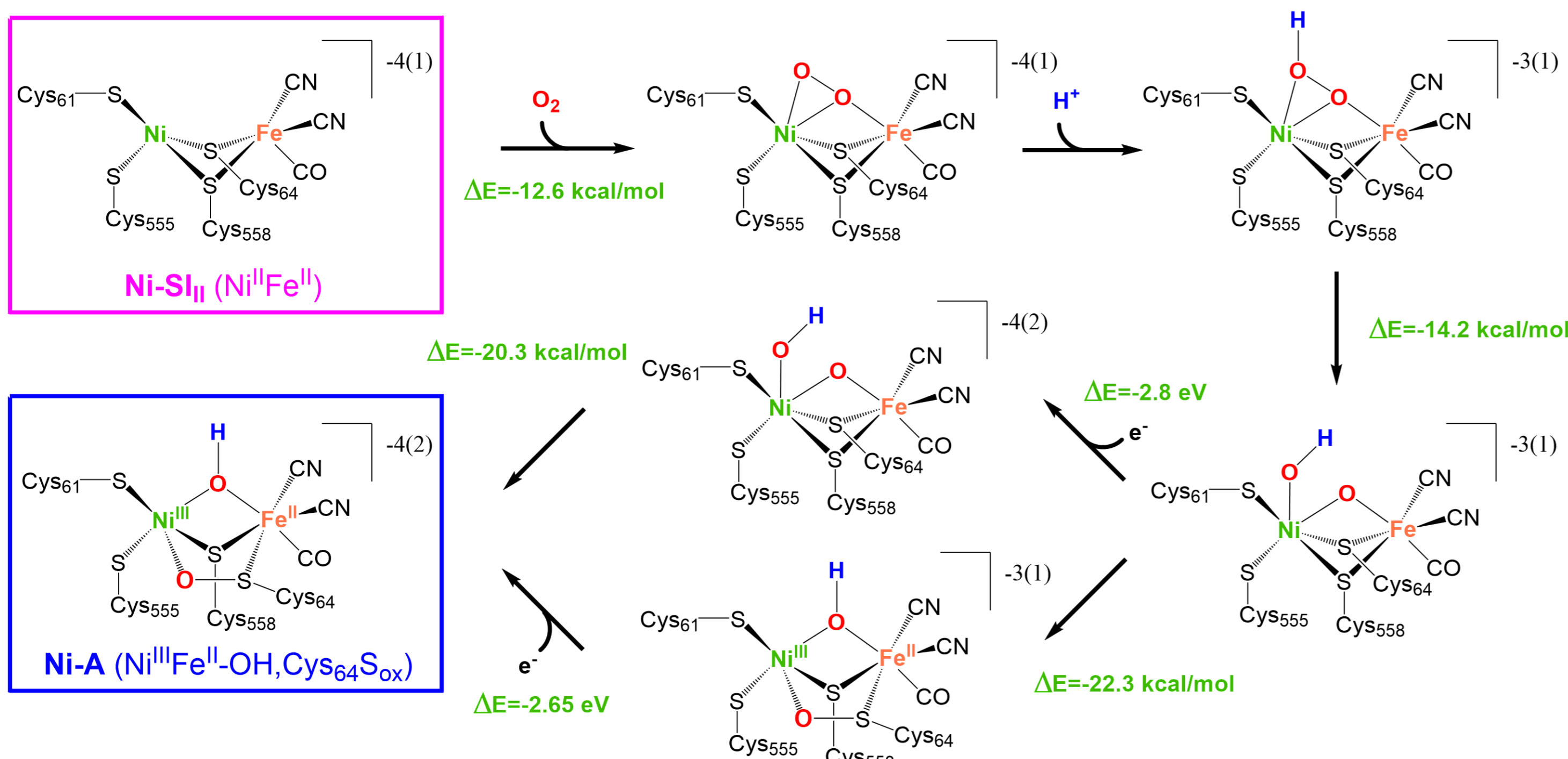
	$E_r$ (kcal/mol)	$\nu_{CO,CN,CN}$ (cm <sup>-1</sup> )	$\Delta\nu_{exp}$ CO, CN, CN (cm <sup>-1</sup> )
(A)	0.0	1944, 2069, 2084	3, 12, 11
(E)	+53.3	1948, 2051, 2074	-1, 30, 21
(F)	+41.1	1936, 2055, 2073	11, 26, 22
(G)	+20.3	1971, 2065, 2098	-24, 16, -3
(H)	+32.7	1942, 2061, 2091	5, 20, 4
Exp		1947, 2081, 2095	

Relative energies and comparison of experimental and predicted IR-frequencies are in agreement with the assignment of Ni-A to a species containing a bridging OH<sup>-</sup> and oxidized Cys64

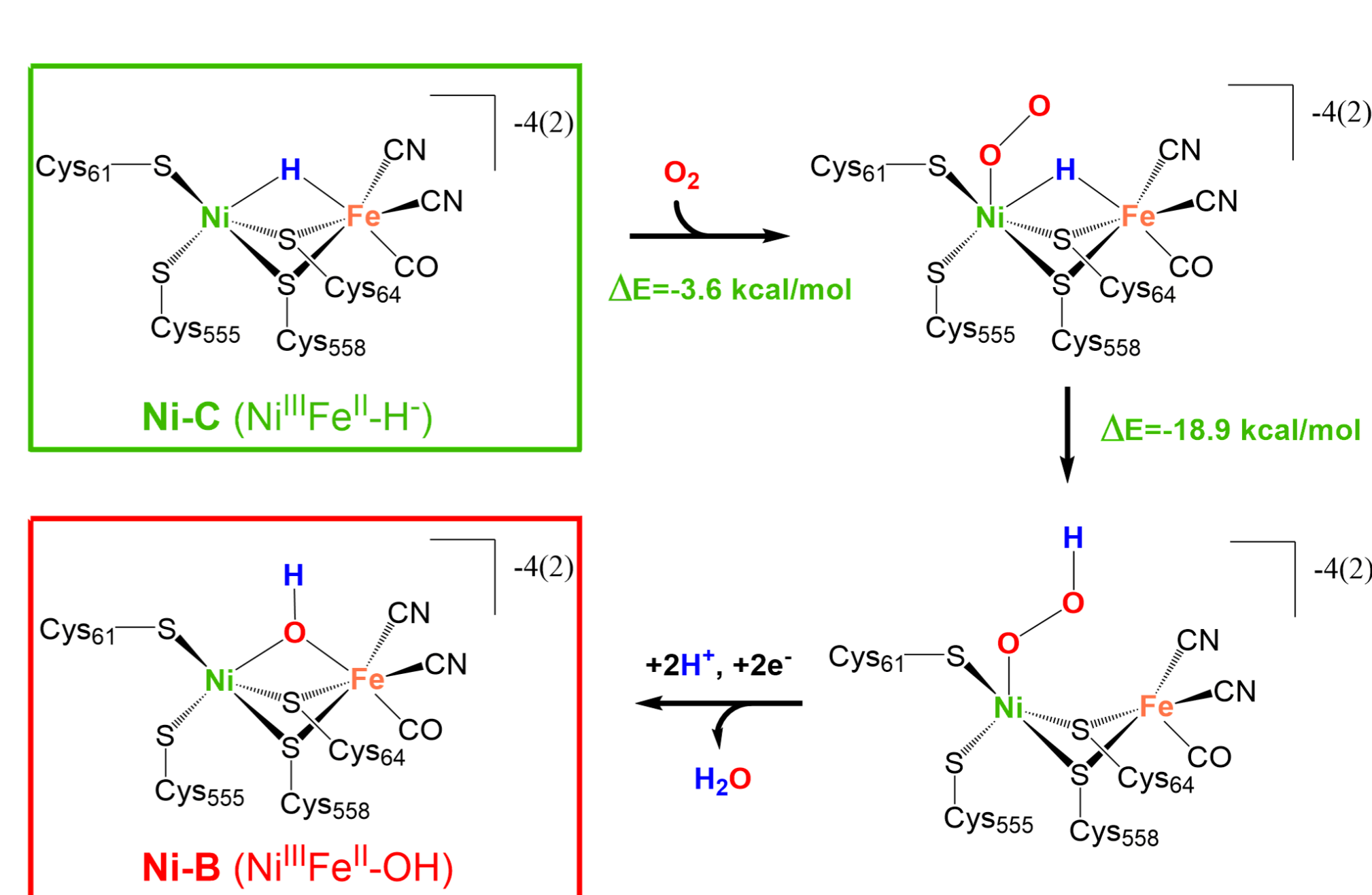
## Oxidation mechanism:

Aerobic oxidation of O<sub>2</sub>-sensitive [NiFe]-Hydrogenases converts the enzyme into two chemically distinct inactive forms; Ni-A and Ni-B. If the [NiFe]-H<sub>2</sub>ase is sufficiently reduced, its treatment with O<sub>2</sub> leads to the formation of a pure Ni-B oxidized state. When a less reduced enzyme is exposed to O<sub>2</sub> the Ni-A is formed in large amounts [2].

Proposed mechanism for the Ni-A formation from Ni-SI<sub>II</sub>:



Proposed mechanism for the Ni-B formation from Ni-C:



## References

- [1] Volbeda A, Martin L, Barbier E, Gutiérrez-Sanz O, De Lacey AL, Liebgott P, Dementin S, Rousset M, Fontecilla-Camps JC (2015) J Biol Inorg Chem 20(1):11-22  
 [2] Volbeda A, Martin L, Cavazza C, Michael M, Faber BW, Roseboom W, Albracht SPJ, Garcin E, Rousset M, Fontecilla-Camps JC (2005) J Biol Inorg Chem (10):239-249