Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supplementary Information

Theoretical investigation of aerobic and anaerobic oxidative inactivation of the [NiFe]-hydrogenase active site

Raffaella Breglia,[a] Claudio Greco,[a] Piercarlo Fantucci,[b] Luca De Gioia[b] and Maurizio Bruschi[a]

[a.] *Department of Earth and Environmental Science, University of Milano Bicocca, Piazza della Scienza 1, 20126 Milan, Italy;*
[b.] *Department of Biotechnology and Biosciences, University of Milano Bicocca, Piazza della Scienza 2, 20126 Milan, Italy.*

Corresponding Author:	Prof. Maurizio Bruschi
e-mail	maurizio.bruschi@unimib.it
tel.	+39-0264482816
fax	+39-0264482890

Table S1 Detailed information of the atoms included in the large (LM) and small (SM) models considered in this work. Hydrogen atoms are not explicitly reported in the list. C, O and N are backbone carbonylic carbon and oxygen, and amidic nitrogen, respectively. Atoms constrained during geometry optimizations are marked with an asterisk. Conversely, atoms in parenthesis indicate atoms at which has been terminated the side chain of the residue.

Residue	**Atoms in LM**	**Atoms in SM**	**Basis set**
$NiFe(CN)_2(CO)$	all	all	def-TZVP
Ile13	C, O and Cα*	none	def-SVP
Glu14	all	side chain (Cγ*)	def-TZVP (carboxylic group), def-SVP (other atoms)
Gly15	N and Cα*	none	def-SVP
Thr17(S)	side chain, Cα*	side chain (Cβ*)	def-SVP
Cys61	all	side chain, Cα*	def-TZVP (side chain), def-SVP (other atoms)
Gly62	zll, H_N*[a]	none	def-SVP
Val63	all	none	def-SVP
Cys64	all	all	def-TZVP (side chain and Cα) def-SVP (other atoms)
Thr65	N, Cα*	none	def-SVP
Val67	side chain, C, O, Cα*	none	def-SVP
His68	side chain, N, Cα*	side chain (Cβ*)	def-SVP
Asp103	side chain, Cα*	side chain (Cβ*)	def-SVP
Arg487	side chain, Cα*	side chain (Cδ*)	def-SVP
Leu490	side chain, Cα, all C atoms*[b]	none	def-SVP
Val507	C, O, Cα*	none	def-SVP
Val508	all	none	def-SVP
Pro509	all	C, O, Cα*	def-SVP
Ser510	all	side chain, N, Cα*	def-SVP
Thr511	N*	none	def-SVP
Asp553	side chain, Cα*	side chain (Cβ*)	def-SVP
Pro554	C, O, Cα*	none	def-SVP
Cys555	all	side chain, Cα*	def-TZVP (side chain and Cα), def-SVP (other atoms)
Ile556	N, C, O*[c], Cα	C, O, Cα*	def-SVP
Ala557	N, C, O*[d], Cα	N, Cα*	def-SVP
Cys558	all,O*[e]	side chain, Cα*	def-TZVP (side chain and Cα), def-SVP other atoms)
Ala559	N, Cα*	none	def-SVP

[a]amidic hydrogen of Gly1062 forms an H-bond with the carbonilic oxygen of Cys16. This H atom has been added at 1.02 Å from N in the C-N-Cα plane, and constrained at that position; [b]all carbon atoms are constrained to the X-ray positions to avoid an unrealistic large rotation of the sidechain during geometry optimization; [c]carbonyl oxygen forms an H-bond with protonated δN of His1561in the α-helix at the C-terminius; [d]carbonyl oxygen forms an H-bond with the amidic hydrogen of His1561 in the α-helix at the C-terminius; [e]carbonyl oxygen forms an H-bond with the amidic hydrogen of Ala1560 in the α-helix at the C-terminius.

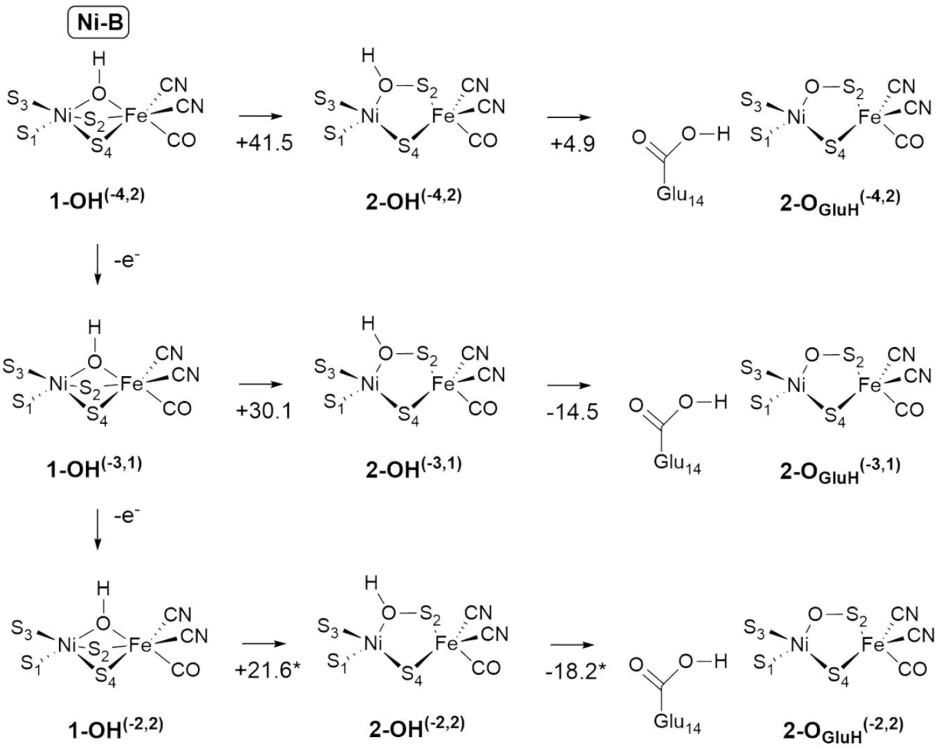


Figure S1 Schematic representation of the insertion of the bridging hydroxide into the Ni-S(Cyss64) bond and subsequent its deprotonation for Ni-B and its mono- and di-oxidized species. Asterisked energy differences are calculated using the LM model in which Asp553 is modelled as protonated. Energy differences are in kcal/mol.

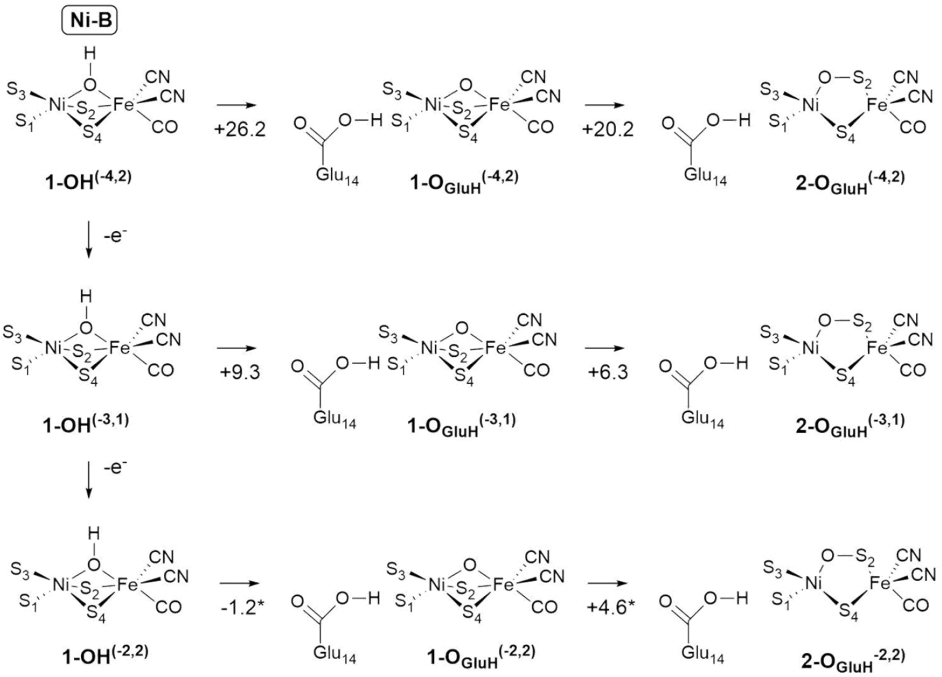


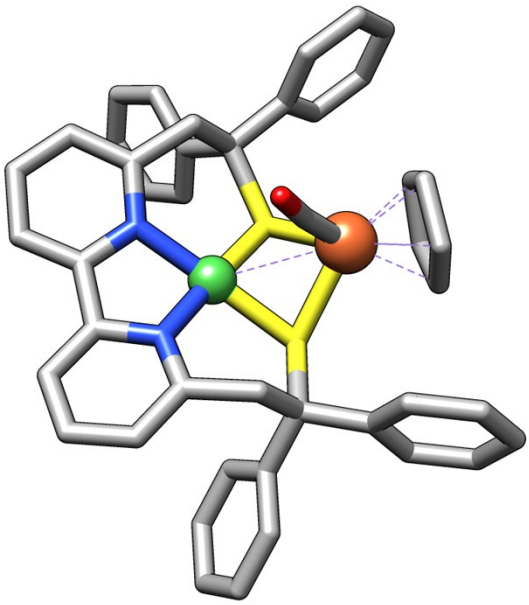
Figure S2 Schematic representation of the bridging hydroxide deprotonation and subsequent insertion of the resulting oxygen atom into the Ni-S(Cyss64) bond for Ni-B and its mono- and di-oxidized species. Asterisked energy differences are calculated using the LM model in which Asp553 is modelled as protonated. Energy differences are in kcal/mol.

Table S2 Energies (in Hartree) of the species investigated in this work, calculated using the large (LM) and the minimal (MM) models at the RI/BP86-defTZVP-SVP level of theory and ε = 4. Asterisked values are calculated using the LM model in which Asp553 is modelled as protonated. Superscripts a and b denote energy values of species in which a water molecule is bounded to the active site or hydrogen bonded, in the nearby of the active site, to Cys61, Cys64 and His68, respectively. Energy calculations using the minimal model have been performed on the geometries optimized for the large model, in which only the peripheral hydrogen atoms have been optimized.

	n-$O_xH_{y(GluH)}$	**LM**	**MM**
		Energy (hartree)	**Energy (hartree)**
x=0, y=0	**$1^{(-5,2)}$**	-10923.00135616	
	$1^{(-4,1)}$	-10922.97718624	-5250,11278739
		-10923.51144427*	
	$1^{(-3,2)}$	-10923.40047778*	-5250,03969357
	$1^{(-2,1)}$	-10923.23732737*	-5249,90433387
x=0, y=0, GluH	**$1_{GluH}^{(-4,2)}$**	-10923.57617184	
	$1_{GluH}^{(-3,1)}$	-10923.51745526	
	$1_{GluH}^{(-2,2)}$	-10923.39455717	
x=0, y=1	**1-$H^{(-5,1)}$**	-10923.59962231	-5250,70947680
	1-$H^{(-4,2)}$	-10923.57923436	-5250,71502246
	1-$H^{(-3,1)}$	-10923.48314292	
	1-$H^{(-2,2)}$	-10923.34491658	
x=1, y=0	**2-$O^{(-3,2)}$**	-10998.10682868	-5325,26468372
x=1, y=0, GluH	**1-$O_{GluH}^{(-4,2)}$**	-10998.81117919	
	1-$O_{GluH}^{(-3,1)}$	-10998.74219173	
	1-$O_{GluH}^{(-2,2)}$	-10999.11140977*	-5325,79273586
	2-$O_{GluH}^{(-4,2)}$	-10998.81117919	-5325,79333079
	2-$O_{GluH}^{(-3,1)}$	-10998.74219173	
	2-$O_{GluH}^{(-2,2)}$	-10999.10411223*	
x=1, y=1	**1-$OH^{(-4,2)}$**	-10998.85294574	
	1-$OH^{(-3,1)}$	-10999.27204376*	-5325,90931511
	1-$OH^{(-2,2)}$	-10999.10945672*	-5325,77949283
	2-$OH^{(-4,2)}$	-10998.78683547	
	2-$OH^{(-3,1)}$	-10998.70903409	
	2-$OH^{(-2,2)}$	-10999.07504974*	
x=1, y=1, GluH	**1-$OH_{GluH}^{(-4,1)}$**	-10999.44914774	-5326,58363969
	1-$OH_{GluH}^{(-3,2)}$	-10999.90908086*	-5326,55181323

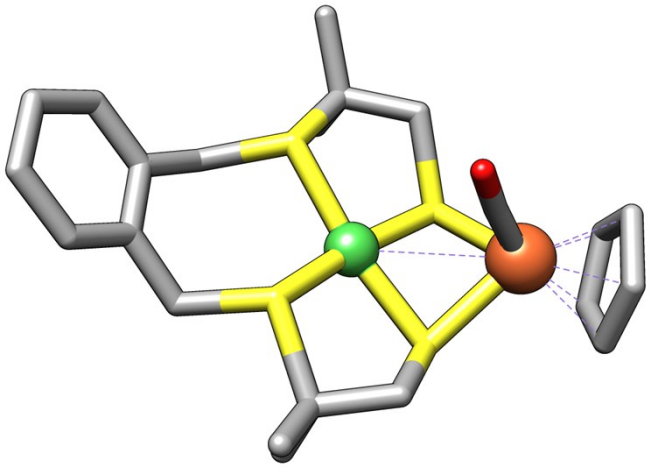
	1-OH$_{GluH}^{(-2,1)}$	-10999.75673618*	-5326,43490937
x=1, y=2	**1-OH$_2^{(-4,1)}$**	-10999.99518453*[a]	-5326,57992780*
		-11000.00185356*[b]	-5326,58066236
	1-OH$_2^{(-3,2)}$	-10999.88641941*[a]	-5326,50609459
		-10999.88859199*[b]	
	1-OH$_2^{(-2,1)}$	-10999.72511944*[a]	-5326,37846656
		-10999.72161288*[b]	
x=2, y=0	**1-O$_2^{(-5,2)}$**	-10923.00135616	
	1-O$_2^{(-4,1)}$	-11073.39949507	
	1-O$_2^{(-4,3)}$	-11073.38708344	
	2-O$_2^{(-4,1)}$	-11073.38406028	
x=2, y=0, GluH	**1-O$_{2GluH}^{(-4,2)}$**	-11074.006430100	
x=2, y=1	**1-O$_2$H$^{(-5,3)}$**	-11074.01736506	-5401,13400432
	1-O$_2$H$^{(-4,2)}$	-11073.98840299	-5401,12843630
	2-O$_2$H$^{(-5,1)}$	-11074.04655622	-5401,15084679
	2-O$_2$H$^{(-4,2)}$	-11074.01466628	-5401,13586822
	3-O$_2$H$^{(-5,1)}$	-11074.05134664	-5401,17451086
	3-O$_2$H$^{(-4,2)}$	-11074.02022569	-5401,16323402
	4-O$_2$H$^{(-5,1)}$	-11074.09656760	-5401,20547557
	4-O$_2$H$^{(-4,2)}$	-11074.05485322	-5401,18755042
	5-O$_2$H$^{(-4,2)}$	-11074.08730907	-5401,22016527
x=2, y=1, GluH	**1-O$_2$H$_{GluH}^{(-4,1)}$**	-11074.57981845	-5401,72796743
	4-O$_2$H$_{GluH}^{(-4,1)}$	-11074,66680201	-5401,80502090
	5-O$_2$H$_{GluH}^{(-3,2)}$	-11074.62851678	-5401,78794879
x=2, y=2	**1-O$_2$H$_2^{(-5,2)}$**	-11074.73153186	
	1-O$_2$H$_2^{(-4,1)}$	-11074.70421349	-5401,84359788
	2-O$_2$H$_2^{(-4,1)}$	-11074.61182261	-5401,73796324
	3-O$_2$H$_2^{(-4,3)}$	-11074.65333119	-5401,78467310
	4-O$_2$H$_2^{(-5,2)}$	-11074. 68691955	
	4-O$_2$H$_2^{(-4,3)}$	-11074.68691955	-5401,82775094
	5-O$_2$H$_2^{(-3,2)}$	-11074.60315643	-5401,73995567
x=2, y=3	**1-O$_2$H$_3^{(-3,1)}$**	-11075.74693496*[a]	
		-11075.76034348*[b]	
	1-O$_2$H$_3^{(-2,2)}$	-11075.58469596*[a]	
		-11075.59377924*[b]	

Table S3. Selected geometrical parameters (distances in Å and angles in degrees) of the [Ni(II)(μ^2,η^2-6,6′-bis(2,2-diphenyl-2-sulfanylethyl)-2,2′-bipyridine)Fe(II)Cp(CO)]$^{+1}$ binuclear cluster calculated using the BP86,[1,2] B3LYP,[2,3] PBE0,[4,5] M06[6] and M06-2X[6] functionals in conjunction with the def-TZVP basis set. The RMSD value has been calculated considering all atoms with the exception of all hydrogens and the four phenyl rings, using as reference the X-ray structure[7]



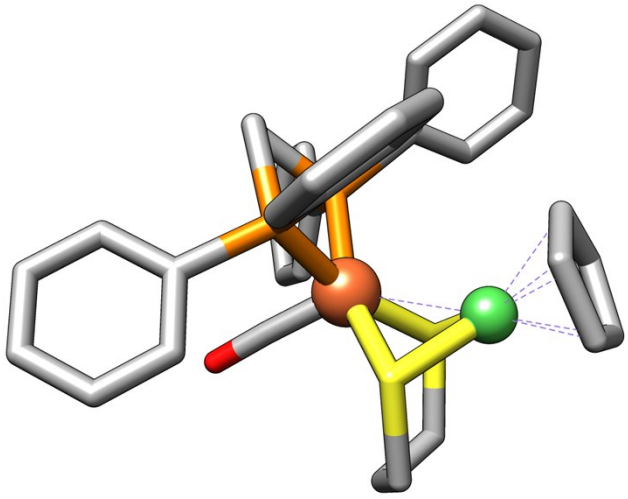
	BP86	**B3LYP**	**PBE0**	**M06**	**M06-2X**	**X-ray**
Ni-Fe	2.750	3.039	2.909	2.845	3.007	2.842
Ni-S	2.215	2.224	2.198	2.210	2.261	2.172
Fe-S	2.329	2.386	2.338	2.343	2.447	2.311
Ni-N	1.919	1.939	1.914	1.930	1.968	1.898
Fe-C(O)	1.759	1.777	1.761	1.782	1.883	1.755
S-Ni-S	77.4	78.9	78.3	78.7	79.5	78.9
S-Fe-S	72.9	72.6	72.8	73.3	72.4	73.5
Ni-S-Fe	74.4	82.1	79.7	77.3	79.3	78.6
RMSD	0.158	0.188	0.116	0.098	0.154	

Table S4. Selected geometrical parameters (distances in Å and angles in degrees) of the [Ni(II)(xbsms)Fe(II)Cp(CO)]$^{+1}$ binuclear cluster calculated using the BP86,[1,2] B3LYP,[2,3] PBE0,[4,5] M06[6] and M06-2X[6] functionals in conjunction with the def-TZVP basis set. The RMSD value has been calculated considering all atoms with the exception of hydrogens using as reference the X-ray structure[8]



	BP86	**B3LYP**	**PBE0**	**M06**	**M06-2X**	**X-ray**
Ni-Fe	3.044	3.131	3.033	3.004	3.027	2.961
Ni-Sb	2.200	2.212	2.186	2.206	2.249	2.168
Ni-St	2.208	2.247	2.212	2.231	2.312	2.180
Fe-S	2.301	2.357	2.308	2.336	2.399	2.302
Fe-C(O)	1.752	1.781	1.764	1.792	1.889	1.761
S-Ni-S	81.4	82.5	82.1	82.3	84.5	80.9
S-Fe-S	76.8	76.5	76.9	77.0	78.2	75.7
Ni-S-Fe	84.9	86.4	85.1	82.8	81.2	82.9
RMSD	0.287	0.363	0.272	0.270	0.319	

Table S5. Selected geometrical parameters (distances in Å and angles in degrees) of the [CpNi(II)(pdt)Fe(II)(CO)(dppe)]$^{+1}$ binuclear cluster calculated using the BP86,[1,2] B3LYP,[2,3] PBE0,[4,5] M06[6] and M06-2X[6] functionals in conjunction with the def-TZVP basis set. The RMSD value has been calculated considering all atoms with the exception of hydrogens using as reference the X-ray structure[9]



	BP86	**B3LYP**	**PBE0**	**M06**	**M06-2X**	**X-ray**
Ni-Fe	2.586	2.639	2.585	2.561	2.719	2.515
Ni-S	2.197	2.220	2.187	2.205	2.291	2.161
Fe-S	2.257	2.288	2.246	2.257	2.337	2.221
Fe-P	2.285	2.344	2.271	2.257	2.359	2.235
Fe-C(O)	1.736	1.761	1.742	1.762	1.898	1.758
S-Ni-S	89.2	89.3	89.6	91.6	91.3	87.4
S-Fe-S	86.2	85.9	86.7	89.1	89.4	90.3
Ni-S-Fe	71.0	71.7	71.3	70.0	72.0	70.0
RMSD	0.226	0.296	0.240	0.209	0313	

Table S6. Mean unsigned error (MUE), with respect to the X-ray values, of the Ni-Fe, Ni-S and Fe-S distances of the NiFe binuclear clusters reported in Tables S3-S5 calculated using the BP86,[1,2] B3LYP,[2,3] PBE0,[4,5] M06[6] and M06-2X[6] functionals, in conjunction with the def-TZVP basis set,.

	BP86	**B3LYP**	**PBE0**	**M06**	**M06-2X**
Ni-Fe	0.082	0.164	0.072	0.031	0.145
Ni-S	0.035	0.056	0.026	0.043	0.116
Fe-S	0.018	0.066	0.019	0.034	0.108

References

1 A. D. Becke, *Phys. Rev. A*, 1988, **38**, 3098–3100.

2 J. P. Perdew, *Phys. Rev. B*, 1986, **33**, 8822–8824.

3 C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785–789.

4 J. P. Perdew, K. Burke and M. Ernzerhof, *Phys. Rev. Lett.*, 1996, **77**, 3865–3868.

5 C. Adamo and V. Barone, *J. Chem. Phys.*, 1999, **110**, 6158–6170.

6 Y. Zhao, D. G. Truhlar, Y. Zhao and D. G. Truhlar, *Theor Chem Acc.*, 2008, **120**, 215–241.

7 D. Brazzolotto, M. Gennari, N. Queyriaux, T. R. Simmons, J. Pécaut, S. Demeshko, F. Meyer, M. Orio, V. Artero and C. Duboc, *Nat. Chem.*, 2016, **8**, 1054–1060.

8 S. Canaguier, M. Field, Y. Oudart, J. Pécaut, M. Fontecave and V. Artero, *Chem. Commun.*, 2010, **46**, 5876.

9 G. M. Chambers, M. T. Huynh, Y. Li, S. Hammes-Schiffer, T. B. Rauchfuss, E. Reijerse and W. Lubitz, *Inorg. Chem.*, 2016, 55, 419–431.