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A Ni(I)Fe(II) analogue of the Ni-L state of the Active Site of the [NiFe] Hydrogenases

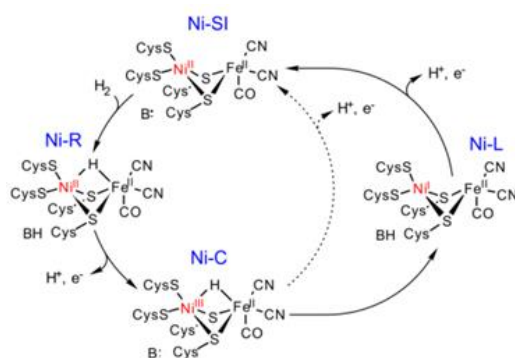
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Ni(L¹)Fe(^tBuNC)₄](PF₆)₂ is a robust Ni^{II}Fe^{II} complex that undergoes a reversible one-electron reduction. Spectroscopic and theoretical studies show that [Ni(L¹)Fe(^tBuNC)₄]⁺ is an unprecedented Ni^IFe^{II} species that reproduces the electronic configuration of the Ni-L state of the [NiFe] hydrogenases.

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The [NiFe]-hydrogenases catalyse the two-electron inter-conversion of two protons and molecular hydrogen.¹ The nature of the Ni-Fe heterobimetallic active site in these enzymes is now established; the Ni centre is co-ordinated by two terminal and two bridging cysteinate donors, which co-ordinate to a Fe centre that is also bound by one carbonyl and two cyanide ligands (Scheme 1). Catalytic H₂ cleavage is associated with changes in the formal oxidation state of the Ni centre while the Fe centre remains in the Fe^{II} state during turnover.^{1c} Three key states have been identified in a catalytic cycle (Scheme 1): Ni-SI, Ni-R and Ni-C. Thus, H₂ reacts with Ni-SI and undergoes heterolytic cleavage to form Ni-R which contains a bridging H⁻ ligand. A co-ordinated Cys ligand may act as an initial proton acceptor for the accompanying proton before its transfer to other bases (B) about the active site.³ The removal of an electron generates the EPR active S = ½ Ni-C state, which can be converted to an EPR-active S = ½ Ni^IFe^{II} state (Ni-L) following the photolysis at low temperatures.⁴ Previously, Ni-L had not been viewed as being catalytically relevant given the conditions required for its formation. However, recent *in situ* IR spectroelectrochemical studies have demonstrated that Ni-L may be generated reversibly in the dark under turnover conditions.² Therefore, the regeneration of the Ni-SI state could occur either directly from the Ni-C state, via the concerted transfer of an electron and a proton, or by oxidation of the Ni-L state. These studies, together with previous DFT calculations,⁵ open up the possibility that separate proton and electron transfer events may be associated with the regeneration of the Ni-SI state from Ni-C, and that these steps may involve the Ni-L state (Scheme 1). Despite the large number of diamagnetic Ni^{II}Fe^{II} complexes that have been prepared as analogues of the [NiFe] hydrogenases,⁶ the syntheses of paramagnetic analogues have proven to be more challenging.⁷ Several Ni^{III}Fe^{III}, Ni^{II}Fe^{III}, Ni^{II}Fe^I and Ni^IFe^I centres have been reported,^{6c,7-8} none of which have succeeded in reproducing the crucial Ni^IFe^{II} and Ni^{III}Fe^{II} states found for the [NiFe] hydrogenases. For example, [(dppe)Ni(μ-pdt)Fe(CO)₃](BF₄)⁷ [dppe = 1,2-bis(diphenylphosphino)ethane, pdt = propane-1,2-dithiolate] possesses a Ni^{II}Fe^I configuration with spin density localized principally on the Fe centre and



Scheme 1: A proposed catalytic cycle for H₂ oxidation by the [NiFe] hydrogenases showing regeneration of Ni-SI directly from Ni-C (dotted line) or via a recently proposed route involving Ni-L (solid line).²

$[(\text{dppe})\text{Ni}(\mu\text{-pdt})\text{Ru}(\text{cymene})]$ ⁹ possesses a Ni^IRu^{II} centre rather than the biologically more relevant Ni^IFe^{II} unit. Given the renewed focus on the role of Ni-L, we report the characterisation of a Ni^IFe^{II} complex ($[\mathbf{1}]^+$) as an analogue of this state. $[\mathbf{1}]^+$ is prepared from the reversible, one-electron reduction of the parent complex $[\mathbf{1}]^{2+}$ (Fig. 1). Our assignment of $[\mathbf{1}]^+$ as a Ni^IFe^{II} centre represents the first analogue of the Ni-L form of the [NiFe] hydrogenases to feature Ni and Fe centres with electronic configurations that mirror those proposed for Ni-L.

Treatment of a solution of $[\text{Ni}(\text{L}^1)]$ ($\text{H}_2\text{L}^1 = \text{N,N}'\text{-diethyl-3,7-diazanonane-1,9-dithiol}$)¹⁰ in acetonitrile with FeCl₂ followed by the addition of four equivalents of ^tBuNC and NH₄PF₆ affords $[\text{Ni}(\text{L}^1\text{N}_2\text{S}_2)\text{Fe}(\text{}^t\text{BuNC})_4](\text{PF}_6)_2$ ($[\mathbf{1}](\text{PF}_6)_2$). $[\mathbf{1}](\text{PF}_6)_2$ is stable at room temperature in air as a solid and in acetonitrile solution for at least 48 h, as monitored by IR spectroscopy. The crystallographic characterization of $[\mathbf{1}](\text{PF}_6)_2 \cdot \text{MeCN}$ shows the Ni centre in an approximate square-planar N_2S_2 environment with Fe having a pseudo-octahedral co-ordination sphere comprised of four ^tBuNC ligands and two S donors derived from $[\text{Ni}(\text{L}^1)]$ (Fig. 1). The Ni N_2S_2 fragment retains the structural features of the $[\text{Ni}(\text{L}^1)]$ precursor,¹¹ the most significant difference being a smaller S(1)-Ni(1)-S(2) angle [81.76(2)°] in $[\text{Ni}(\text{L}^1)]$ relative to that in $[\mathbf{1}]^{2+}$ [84.20(2)°]. This difference may reflect the steric demands of the co-ordinated $[\text{Fe}(\text{}^t\text{BuNC})_4]^{2+}$

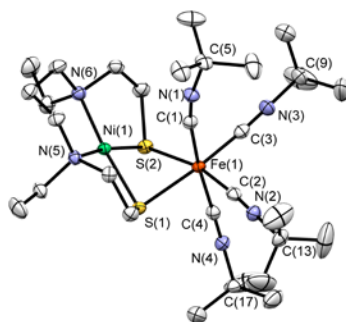


Fig. 1: X-Ray structure of the cation $[1]^{2+}$ in $[1](PF_6)_2$ with 50% probability thermal ellipsoids.

fragment in $[1]^{2+}$. The equatorial ${}^t\text{BuNC}$ ligands defined by C(2) and C(3) bind to Fe(1) in an essentially linear mode with Fe(1)-C-N angles of $175.3(2)^\circ$ and $175.1(2)^\circ$, respectively. In contrast the axial ${}^t\text{BuNC}$ ligands, defined by C(1) and C(4), co-ordinate in a bent geometry with Fe(1)-C-N angles of $170.0(2)$ and $172.9(2)^\circ$, respectively. The Ni(1)-C(1) and Ni(1)-C(4) distances [$2.951(2)$ and $4.164(2)$ Å, respectively] are significantly greater than the Fe(1)-C(1) and Fe(1)-C(4) distances [$1.890(2)$ and $1.888(2)$ Å, respectively]. Thus, the axial ${}^t\text{BuNC}$ ligands do not appear to adopt bridging modes between the Ni and Fe centres in $[1]^{2+}$ and the non-linear binding mode of these ligands about Fe(1) may result from inter- and intra-molecular interactions due to crystal packing (Fig. S1). The Ni(1)-Fe(1) distance [$2.9898(7)$ Å] compares well with that in the inactive oxidised form of [NiFe] hydrogenase from *Desulfovibrio gigas* (2.9 Å)¹² and is significantly longer than that found in the Ni-R form from *Desulfovibrio vulgaris* Miyazaki F (2.57 Å)^{3a} that both contain Ni and Fe in formal M^{II} oxidation states.

The cyclic voltammogram of $[1](PF_6)_2$, recorded at 298K in MeCN containing 0.2 M $[\text{N}^t\text{Bu}_4][\text{BF}_4]$ as supporting electrolyte, shows a reduction process at $E_{1/2} = -1.39$ V vs Fc^+/Fc that is reversible over the range of scan rates employed in the experiment (20 – 300 mVs^{-1} , Figs. S2 and S3). The cyclic voltammogram of $[\text{Ni}(\text{L}^1)]$ recorded under the same conditions reveals a reduction process at $E_p^c = -2.35$ V vs Fc^+/Fc (Fig S4), assigned to the reduction of $[\text{Ni}(\text{L}^1)]$ to the formal Ni^{I} state on the

basis of comparisons with previously reported NiN_2S_2 complexes possessing similar co-ordination spheres.¹³ The shift of *ca.* +1 V for the reduction of $[\mathbf{1}](\text{PF}_6)_2$ relative to that of $[\text{Ni}(\text{L}^1)]$ is consistent with the formation of a Lewis base Lewis acid adduct between $[\text{Ni}(\text{L}^1)]$ and $[\text{Fe}(\text{}^t\text{BuNC})_4]^{2+}$; *ca.* +0.5 V shifts in potential have been observed previously for $(\text{NiN}_2\text{S}_2)\text{W}(\text{CO})_4$ relative to their parent NiN_2S_2 complexes.¹³ UV/Vis spectroelectrochemistry indicates that $[\mathbf{1}]^+$ decomposes at temperatures above 273 K and that cooling to 243K is required to ensure the quantitative regeneration of $[\mathbf{1}]^{2+}$ (Fig. S5, Table S1). On cooling to 243 K the cyclic voltammogram of $[\mathbf{1}](\text{PF}_6)_2$ becomes electrochemically irreversible (Fig. S6) and the controlled potential electrolysis of $[\mathbf{1}](\text{PF}_6)_2$ at -1.6 V vs Fc^+/Fc at 243K confirms that a one-electron reduction process accompanies the formation of $[\mathbf{1}]^+$. The cyclic voltammograms of $[\mathbf{1}](\text{PF}_6)_2$ and $[\mathbf{1}]^+$ at 243K exhibit similar profiles confirming the stability of $[\mathbf{1}]^+$ under the conditions and timescale of the experiment (Fig. S7).

The IR spectra of $[\mathbf{1}](\text{PF}_6)_2$ and $[\mathbf{1}]^+$ in MeCN solution are shown in Fig. 2. Each spectrum exhibits four bands assigned to the C-N stretches of the ${}^t\text{BuNC}$ ligands. In $[\mathbf{1}]^{2+}$ these bands occur at frequencies typical of isonitrile ligands bound in a terminal mode to transition metal centres.¹⁴ The overall shift of the bands to lower frequencies following the reduction of $[\mathbf{1}]^{2+}$ to $[\mathbf{1}]^+$ is consistent with an increase in the electron density about the Ni-Fe core and a corresponding increase in π -back-donation into the ${}^t\text{BuNC}$ units. A C-N stretching band at 1857 cm^{-1} in the IR spectrum of $[\mathbf{1}]^+$ in MeCN solution suggests that one terminal, apical ${}^t\text{BuNC}$ ligand moves to a bridging mode between the Ni and Fe centres (Fig. 2) following the reduction of $[\mathbf{1}]^{2+}$; a similar bridging mode is found in $[\text{Fe}_2(\text{pdt})(\text{MeNC})_7](\text{PF}_6)_2$ where one MeNC ligand bridges between the two Fe centres.¹⁵

The X-band EPR spectrum of electrochemically generated $[\mathbf{1}]^+$ recorded at 77 K in MeCN / 0.2 M $[\text{N}^n\text{Bu}_4][\text{BF}_4]$ (Fig. 3a) shows striking similarities to those of $\text{Ni}^{\text{I}}\text{N}_2\text{S}_2$ complexes ($S = \text{thiolato}$, thioether or sulfonato , $N = \text{amine donors}$; $g_{\parallel} = 2.18 - 2.25$; $g_{\perp} = 2.057-2.071$), generated by

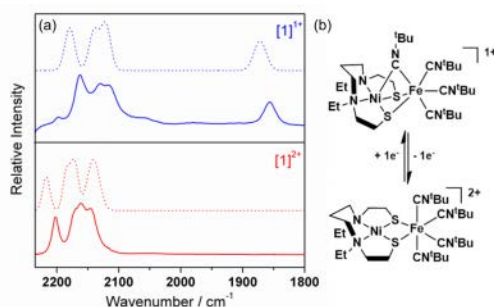


Fig. 2: (a) solution IR spectra of **[1]**(PF₆)₂ (2200, 2170, 2161 and 2144 cm⁻¹) and **[1]⁺** (2162, 2129, 2116 and 1857 cm⁻¹) recorded in MeCN (solid lines) and DFT calculated spectra (dotted lines; 2216, 2183, 2169, 2139 cm⁻¹ for **[1]²⁺** and 2179, 2140, 2121, 1872 for **[1]⁺**). (b) proposed rearrangement supported by DFT calculations.

chemical reduction of their Ni^{II} counterparts,¹⁶ and, crucially, is substantially different to those of Fe^I centres including [Fe(CO)₃(PPh₃)₂]⁺ ($g_{xx} = 2.053$, $g_{yy} = 2.090$, $g_{zz} = 2.001$)¹⁷ and [(dppe)Ni(μ-pdt)Fe(CO)₃]⁺ ($g_{xx} = 2.052$, $g_{yy} = 2.050$, $g_{zz} = 2.005$ for one isomer).⁷ Thus, the EPR spectroscopic data are consistent with a formal Ni^IFe^{II} unit in **[1]⁺** where the Ni^I centre adopts a d⁹, S = ½ configuration in which the unpaired electron resides in d-orbital orientated in the equatorial plane of the Ni^IN₂S₂ unit with associated spin Hamiltonian parameters $g_{zz} > g_{xx} \approx g_{yy} > g_e$.¹⁸ In contrast, the Ni-L form of the [NiFe] hydrogenases is characterised by a rhombic EPR spectrum ($g_{11} = 2.30$, $g_{22} = 2.12$ and $g_{33} = 2.05$)¹⁹ that may be viewed as resulting from the re-hybridization of the Ni d_{x²-y²} and d_{z²} orbitals in Ni-C where one hybrid contributes to a Ni-Fe bond.²⁰ The UV/vis spectrum of **[1]⁺** (Table S1 and Fig. S5) shows bands at 400 (3900), 490 (1760), 520 (1500), 598 (1100) and 720 nm (380 M⁻¹ cm⁻¹) that are consistent with those in the UV/vis spectra of other well-defined Ni^I complexes.²¹

In order to support the Ni^IFe^{II} assignment proposed for **[1]⁺**, we conducted density functional theory (DFT) calculations on the full structures of **[1]^{2+/+}**. The calculated structure of **[1]²⁺** [Fig. S8(a)] reproduces the principal features of the experimentally determined structure (Fig. 1); the average Fe-C distances are *ca.* 0.02 Å shorter and the Ni-S and Fe-S distances are *ca.* 0.04 Å longer in the calculated structure of **[1]²⁺** (Table S3). The principal differences between the calculated and

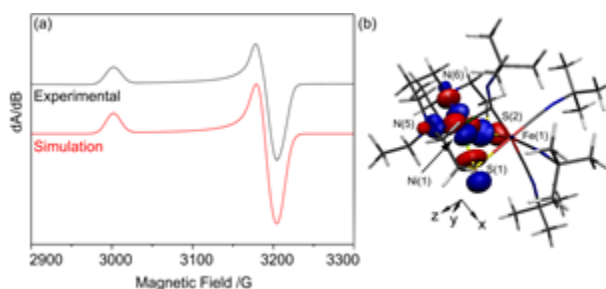


Fig. 3: (a) X-band EPR spectrum of $[1]^+$ as a solution in MeCN/0.2 M $[N^tBu_4][BF_4]$ at 77 K.

Experimental (black) and simulated (red) spectrum, simulated using the spin Hamiltonian

parameters $g_{11} = 2.210$, $g_{22} = g_{33} = 2.074$ ($W_{11} = 18$, $W_{22} = 16$, $W_{33} = 17$ G); (b) The Kohn-Sham

SOMO of $[1]^+$ plotted with an isosurface value of $0.05 \text{ e}\text{\AA}^{-3}$.

experimental structures are (i) a relaxation of the dihedral angle defined by the S(1)-Ni(1)-S(2) and S(1)-Fe(1)-S(2) planes [$117.94(3)^\circ$ and 128.3° , in the calculated and experimental structures, respectively], (ii) an increase in the Ni(1)-Fe(1) distance of *ca* 0.2 \AA in the calculated relative to the experimental structure of $[1]^{2+}$ [Ni(1)-Fe(1) = 3.209 \AA and $2.9896(4) \text{ \AA}$ for the calculated and experimental structures, respectively, Table S2], and (iii) an increase in the of the C-Fe(1)-C and Fe(1)-C-N angles for the axial t BuNC ligands defined by C(1) and C(4) (Table S2). The unscaled²² calculated IR spectrum of $[1]^{2+}$ possesses four bands in the C-N stretching region at 2216 , 2183 , 2169 and 2139 cm^{-1} that compare well with the experimental stretching frequencies (Fig. 2). Thus, the close correspondence between the calculated and experimental structures, and IR spectra suggest that the DFT calculations provide a reasonable description of the geometric and electronic structure of $[1]^{2+}$. The composition²³ of the HOMO in $[1]^{2+}$ shows that it is largely metal-centred [59.3% Ni d_z , 0.6% Ni d_{xz} , 9.7% Fe $d_{x^2-y^2}$, 1.2% Fe d_{xz} , S 25.8%, N(5)+N(6) 1.2%, Fig. S9] The Mayer bond order²⁴ between the Ni(1) and Fe(1) centres (0.04) derived from the DFT calculations suggests there is no formal metal-metal bond in $[1]^{2+}$. Overall the description of the electronic structure of $[1]^{2+}$ is consistent with an $S = 0$ $Ni^{II}Fe^{II}$ centre in $[1]^{2+}$.

The calculated structure of $[\mathbf{1}]^+$ suggests that significant changes in geometry about the Ni(1) and Fe(1) centres accompany the reduction of $[\mathbf{1}]^{2+}$ [Fig. S8(b) and Table S3] These include a marked decrease in the dihedral angle between the S(1)-Ni(1)-S(2) and S(1)-Fe(1)-S(2) planes (94.1° and 128.3° , for $[\mathbf{1}]^+$ and $[\mathbf{1}]^{2+}$, respectively) and a shortening of the Ni(1)-Fe(1) distance [Ni(1)-Fe(1) = 2.616 \AA and 3.209 \AA for $[\mathbf{1}]^{1+}$ and $[\mathbf{1}]^{2+}$, respectively], which compares well with that calculated for models of the active site of the Ni-L form.²⁰ On the reduction of $[\mathbf{1}]^{2+}$ one axial ^tBuNC ligand, defined by C(1), moves to a bridging mode between the Fe(1) and Ni(1) centres with Fe(1)-C(1) and Ni(1)-C(1) distances of 1.942 and 2.018 \AA , respectively. The adoption of a bridging mode for this ligand is accompanied by a significant bend in the backbone of the ligand [C(1)-N(1)-C(5) = 140.9°], which is commonly observed for bridging isocyanides.²⁵ The calculated IR spectrum for $[\mathbf{1}]^+$ shows three intense C-N stretches at 2179 , 2140 and 2121 cm^{-1} for the terminal isocyanides and a single band at 1872 cm^{-1} for the C-N stretching mode of the bridging ^tBuNC ligand. This calculated spectrum shows close correspondence to the experimental IR spectrum of $[\mathbf{1}]^+$ (Fig. 2) and strongly supports a structural rearrangement in which a terminal ^tBuNC ligand moves to a bridging mode on the reduction of $[\mathbf{1}]^{2+}$. This structural rearrangement may also underpin the differences in profiles of the cyclic voltammograms of $[\mathbf{1}](\text{PF}_6)_2$ recorded at 298 K (Fig. S2) and 243 K (Fig. S6). The rate of the suggested structural rearrangements for $[\mathbf{1}]^{2+/+}$ may be slowed at 243 K with the consequent loss of electrochemical reversibility for the $[\mathbf{1}]^{2+/+}$ process at 243 K . We are unable to determine the precise mechanism that gives rise to the voltammetric profile *i.e.* whether electron transfer precedes structural rearrangement or *vice versa*. However, the results of the UV/vis spectroelectrochemical experiments clearly show that the process is chemically reversible at 243 K over the timescale of this experiment.

The SOMO of $[\mathbf{1}]^+$ possesses 60.8% Ni d_{xy} , 1.3% Ni d_{xz} , 1.1% Ni d_{yz} S 21.7%, N(5)+N(6) 10.2% character and is essentially localized on the NiN_2S_2 unit (Fig. 3b). The calculated EPR spin

Hamiltonian parameters using the BP86 functional ($g_{zz} = 2.174$, $g_{yy} = 2.079$, $g_{xx} = 2.070$, Table S2) reproduce the approximately axial nature of the frozen solution EPR spectrum of $[\mathbf{1}]^+$ (Fig. 3a). We note that the DFT calculations underestimate the largest g-shift ($g_{zz} = 2.174$ calc. vs $g_{11} = 2.210$). Such underestimations (by up to 30%) have been observed previously for various metal centres including Ni^{I} , and these underestimations have been attributed partly to overestimations in spin delocalization into ligand-based orbitals in the calculated electronic structures.^{20,26} Thus, these results, together with the excellent agreement between the calculated and experimental IR spectra, support a $\text{Ni}^{\text{I}}\text{Fe}^{\text{II}}$ description for $[\mathbf{1}]^+$ where the unpaired electron is essentially localised in the d_{xy} orbital of a d^9 Ni^{I} centre. The calculated Ni-Fe Mayer bond order increases from 0.04 in $[\mathbf{1}]^{2+}$ to 0.20 in $[\mathbf{1}]^+$ indicating the development of a Ni-Fe interaction but not a direct bond. In contrast, DFT calculations on models of the Ni-L state possess Ni-Fe bond orders of *ca.* 0.40 supporting the formation of a metal-metal bond in these centres.²⁰ The absence of a formal Ni-Fe bond in $[\mathbf{1}]^+$ is not surprising given the additional fourth ^tBuNC ligand in the co-ordination sphere of Fe^{II} which occupies a bridging position between the Ni and Fe centres; this site is vacant in structures proposed for Ni-L.

Ni-L reacts with CO and converts to the paramagnetic Ni-CO state which features a CO ligand bound to the Ni^{I} centre.²⁷ Thus, we examined the reactivity of $[\mathbf{1}]^{2+}$ and $[\mathbf{1}]^+$ towards CO. Whereas $[\mathbf{1}]^{2+}$ does not react with CO, a solution of $[\mathbf{1}]^{1+}$ chemically generated from $[\mathbf{1}](\text{PF}_6)$ with $[\text{Cp}^*\text{Co}]$ readily reacts with CO at 243 K, as monitored by IR spectroscopy (Fig. S10). Several new bands develop in the C-O and C-N stretching region and the frozen solution EPR spectrum exhibits multiple low field features (Fig. S10) suggesting the formation of multiple products, which proved intractable.

In conclusion, $[\mathbf{1}]^{2+}$ has been prepared and structurally characterized as its $[\mathbf{1}](\text{PF}_6)_2$ salt. The electrochemical one-electron reduction of $[\mathbf{1}]^{2+}$ generates paramagnetic $[\mathbf{1}]^+$ which has been

characterized by IR, UV/vis and EPR spectroscopies. DFT calculations reproduce the principal features of the IR spectrum of $[1]^+$ and, in contrast to Ni-L, $[1]^+$ does not contain a formal Ni-Fe bond. Rather the formation of $[1]^+$ may be associated with a structural rearrangement that incorporates a bridging $^t\text{BuNC}$ ligand between the Ni and Fe centres. The frozen solution X-band EPR spectrum of $[1]^+$ and DFT calculated spin Hamiltonian parameters are consistent with a SOMO that is largely localized at the NiN_2S_2 core in a Ni d_{xy} orbital. Thus, the experimental and theoretical data supports the assignment of $[1]^+$ to a mixed-valence $\text{Ni}^{\text{I}}\text{Fe}^{\text{II}}$ state. In this respect $[1]^+$ represents the first example of an Ni-Fe analogue of the active site of the $[\text{NiFe}]$ hydrogenases that reproduces the formal oxidation and spin states of the metal centres in the Ni-L form.

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