

Enzyme Models | Hot Paper |

Synthetic Active Site Model of the [NiFeSe] Hydrogenase

Claire Wombwell and Erwin Reisner*^[a]

Dedicated to Professor Stephen J. Lippard on the occasion of his 75th birthday

Abstract: A dinuclear synthetic model of the [NiFeSe] hydrogenase active site and a structural, spectroscopic and electrochemical analysis of this complex is reported. $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ ($\text{H}_2\text{S}_2\text{Se}_2' = 1,2\text{-bis}(2\text{-thiabutyl-}3,3\text{-dimethyl-}4\text{-selenol})\text{benzene}$) has been synthesized by reacting the nickel selenolate complex $[\text{Ni}(\text{S}_2\text{Se}_2)']$ with $[\text{Fe}(\text{CO})_3\text{bda}]$ ($\text{bda} = \text{benzylideneacetone}$). X-ray crystal structure analysis confirms that $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ mimics the key structural features of the enzyme active site, including a doubly bridged heterobimetallic nickel and iron center with a selenolate terminally coordinated to the nickel center. Compari-

son of $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ with the previously reported thiolate analogue $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$ ($\text{H}_2\text{S}_4' = \text{H}_2\text{xbsms} = 1,2\text{-bis}(4\text{-mercapto-}3,3\text{-dimethyl-}2\text{-thiabutyl})\text{benzene}$) showed that the selenolate groups in $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ give lower carbonyl stretching frequencies in the IR spectrum. Electrochemical studies of $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ and $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$ demonstrated that both complexes do not operate as homogenous H_2 evolution catalysts, but are precursors to a solid deposit on an electrode surface for H_2 evolution catalysis in organic and aqueous solution.

Introduction

The depletion of fossil fuel reserves, the increasing levels of atmospheric CO_2 , and the need for energy security drive the development of new approaches to produce a renewable energy vector such as H_2 .^[1] Inexpensive, stable, and efficient H_2 generation catalysts are needed to produce sustainable H_2 from water in the long term.^[2] Hydrogenases are reversible H_2 production catalysts and display remarkably high turnover frequencies of over 10^3 s^{-1} at a small overpotential.^[3] This incredible activity is achieved using the abundant metals nickel and iron in the hydrogenase active site.^[4] [NiFeSe] hydrogenases are a subclass of the [NiFe] hydrogenases, where a selenocysteine (Sec) residue is terminally coordinated to the nickel center instead of a cysteine (Cys) in the enzyme active site (Figure 1).^[5] [NiFeSe] hydrogenases have emerged as particularly suitable catalysts for H_2 evolution,^[6] because they exhibit high catalytic activities for H_2 generation in the presence of H_2 and fast reactivation from O_2 inactivation when compared with other hydrogenases.^[7] These advantageous properties make [NiFeSe] hydrogenases attractive for use in H_2O splitting sys-

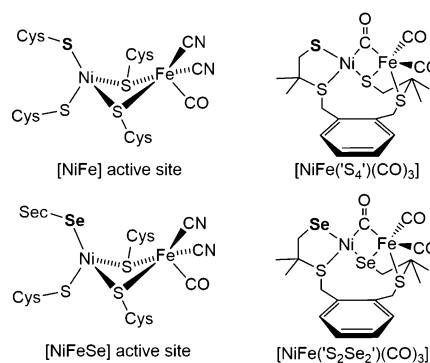


Figure 1. Representation of the active-site structures of a [NiFe] and [NiFeSe] hydrogenase in a reduced, active (Ni-SI) state and the corresponding synthetic models $[\text{NiFe}(\text{S}_4')(\text{CO})_3]$ ^[15d] and $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ (this work).

tems, and have allowed for their exploitation in a number of efficient photocatalytic H_2 production schemes.^[7e,8]

As with other selenium containing enzymes,^[9] it is still unclear what role selenium plays in the [NiFeSe] hydrogenases.^[6] The Sec residue may affect the electronic and steric properties of the bimetallic core at the active site. Crystallographic evidence suggests that the Sec residue in the [NiFeSe] hydrogenase behaves as a proton relay during catalytic H_2 cycling, carrying protons to and from the active site.^[5] It is the Cys residue in the same position in the [NiFe] hydrogenase that was proposed to be the proton relay (Figure 1).^[10] The unique reactivity of the [NiFeSe] hydrogenase with O_2 may be the reason for its fast reactivation from O_2 inactivation. When a conventional [NiFe] hydrogenase reacts with O_2 , the nickel center is oxidized to nickel(III) and an oxygen containing ligand takes the bridging position between the nickel and the iron centers.^[11] In the O_2 oxidized [NiFeSe] hydrogenase, however, the nickel center

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is not oxidized and no bridging ligand is observed between the two metal centers.^[7g,12] Crystallographic evidence suggests that it is the Sec selenium and, in some cases, Cys sulfur that is oxidized in [NiFeSe] hydrogenases.^[12c,d,13]

It has been well-established that the protein structure surrounding an active site affects the reactivity of an enzyme,^[14] and biomimetic molecules can be employed to learn about the structural and functional properties of the active site.^[15] Our aim is to explore the effect of selenium on the enzyme active site using small molecule model chemistry.

A large number of dinuclear models of the active site of the conventional [NiFe] hydrogenases have previously been reported following the determination of the X-ray crystal structure of the enzyme.^[11c,15d,f,h,m,n,16] Several structural and functional [NiFe] hydrogenase models have been prepared using the nickel precursor complex [Ni('S₄')] (H₂'S₄' = H₂xbsms = 1,2-bis(4-mercapto-3,3-dimethyl-2-thiabutyl)benzene).^[15d,f,16b,17] [Ni('S₄')] was initially used as precursor to assemble a number of thiolate bridged [NiFe] complexes^[16b] and dinuclear [NiRu] complexes.^[17b-d,f] The [NiRu] complexes were some of the first functional hydrogenase models reported to catalyze H₂ production.^[17b-d,f] Since then, two structural and functional [NiFe] hydrogenase models that use [Ni('S₄')] as a precursor have been reported.^[15d,f] One of these, the asymmetrical [NiFe] complex [NiFe('S₄')CO₃], is the only dinuclear [NiFe] hydrogenase model that contains a thiolate donor terminally bound to the nickel center (Figure 1).^[15d]

We have recently reported on a series of nickel complexes as structural models of the nickel center in the active sites of the [NiFeSe] hydrogenases.^[18] Herein, we report a dinuclear [NiFeSe] hydrogenase active site model, which includes an iron carbonyl to replicate the core features of the enzyme active site. The synthesis, characterization, and activity of [NiFe('S₂Se₂')CO₃] is reported and it has been compared with the previously reported^[15d] thiolate analogue [NiFe('S₄')CO₃] to determine the influence of the chalcogenate donor on the properties of the complex (Figure 1). A detailed structural, spectroscopic, and electrochemical analysis as well as a comparison with the hydrogenase active site is presented.

Results and Discussion

Synthesis and characterization of [NiFe('S₂Se₂')CO₃]

[NiFe('S₂Se₂')CO₃] was synthesized following the procedure shown in Figure 2. Bis(3-chloro-2,2-methyl-1-thiaprolyl)-o-xylene^[17a] was reacted with two equivalents of selenourea in ethanol at room temperature to give the selenouronium compound 'S₂Se₂'_{pre}. This ligand precursor was isolated as a white powder in 61% yield and characterized by ¹H and ¹³C NMR spectroscopy, mass spectrometry, and elemental analysis (Supporting Information, Figures S1–S5). [Ni('S₂Se₂')] was prepared by refluxing [Ni(acac)₂] (acac = acetylacetonato) with one equivalent of 'S₂Se₂'_{pre} and two equivalents of NMe₄OH·5H₂O as a base under inert conditions for 1 h in ethanol. The solvent volume was decreased to precipitate the product as a green solid, which was separated by filtration and recrystallized by

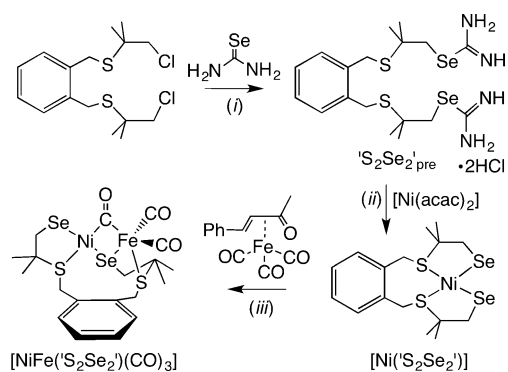


Figure 2. Synthesis of the 'S₂Se₂' ligand precursor 'S₂Se₂'_{pre}, the mononuclear Ni complex [Ni('S₂Se₂')], and the [NiFeSe] hydrogenase active site mimic [NiFe('S₂Se₂')CO₃]. Conditions: i) ethanol, RT, 61% yield; ii) 2 equiv NMe₄OH·5H₂O, ethanol, reflux, 83% yield; iii) dichloromethane, RT, 43% yield.

slow diffusion of hexane into a dichloromethane solution of the complex to give [Ni('S₂Se₂')] in 83% yield. The green complex was characterized by ¹H and ¹³C NMR, IR, and electronic absorption spectroscopy, mass spectrometry, elemental analysis, and single-crystal X-ray diffraction (Supporting Information, Figures S6–S10). Reaction of [Ni('S₂Se₂')] with one equivalent of [Fe(CO)₃(bda)] (bda = benzylideneacetone) in dichloromethane readily formed the red solid [NiFe('S₂Se₂')CO₃] at room temperature, which was isolated in 43% yield. [NiFe('S₂Se₂')CO₃] was characterized using ¹H and ¹³C NMR, IR, and electronic absorption spectroscopy, mass spectrometry, elemental analysis, and single-crystal X-ray diffraction (Supporting Information, Figures S11–S16).

Structural characterization

Single crystals of [Ni('S₂Se₂')] were grown by liquid diffusion of hexane into a saturated dichloromethane solution of the complex and the X-ray crystal structure is shown in Figure 3A. Complex [Ni('S₂Se₂')] crystallizes in the space group P2₁/n with two crystallographically independent molecules per asymmetric unit. Selected distances and angles for [Ni('S₂Se₂')] and the previously reported [Ni('S₄')]^[17a] are summarized in Table 1.

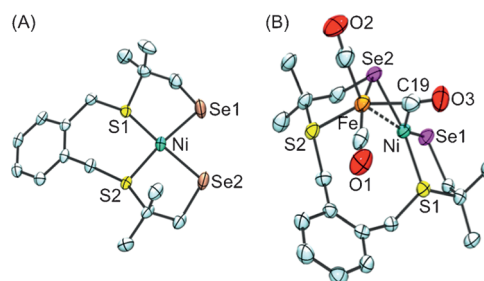


Figure 3. Single-crystal X-ray structures of A) [Ni('S₂Se₂')] (R1 = 3.11%) and B) [NiFe('S₂Se₂')CO₃] (R1 = 2.98%). Ellipsoids are set at 50% probability; hydrogen atoms have been omitted for clarity. The atom labeling shows all atoms except for carbon. One of two crystallographically independent molecules is shown for [Ni('S₂Se₂')]. A disordered solvent pentane molecule is omitted in the structure of [NiFe('S₂Se₂')CO₃].

Table 1. Selected bond distances and angles for [Ni('S₂Se₂')] and [Ni('S₄')] model complexes.^[a]

[Ni('S ₂ Se ₂ ')] Distance [Å]	[Ni('S ₄ ')] Distance [Å]
Ni–Se1 2.3023(7)	Ni–S'1 2.1802(6)
Ni–Se2 2.2872(7)	Ni–S'2 2.1869(6)
Ni–S1 2.192(1)	Ni–S1 2.1909(6)
Ni–S2 2.184(1)	Ni–S2 2.1756(5)

[Ni('S ₂ Se ₂ ')] Angle [°]	[Ni('S ₄ ')] Angle [°]
Se1–Ni–Se2 84.21(2)	S'1–Ni–S'2 84.05(2)
Se2–Ni–S2 89.69(3)	S'2–Ni–S2 87.11(2)
S1–Ni–S2 97.16(4)	S1–Ni–S2 100.75(2)
Se1–Ni–S1 88.93(3)	S'1–Ni–S1 87.92(2)
S1–Ni–Se2 173.12(4)	S1–Ni–S'2 171.45(2)
Se1–Ni–S2 167.31(4)	S'1–Ni–S'2 170.72(2)

[a] Distances and angles for one of two molecules in the asymmetric unit in both cases; S = thioether, S' = thiolate donor.

Crystal data and refinement details for [Ni('S₂Se₂')] are given in the Supporting Information, Table S1. [Ni('S₂Se₂')] contains nickel(II) coordinated to two selenolate and two thioether donors with square-planar geometry around the nickel center. The bond distances are as expected: the nickel selenolate distances of 2.295(8) Å in [Ni('S₂Se₂')] are longer than the nickel thiolate distances of 2.184(3) Å in [Ni('S₄')] model complexes,^[17a] as observed with other nickel thiolate/selenolate complexes.^[18] The five-membered rings in [Ni('S₂Se₂')] constrain the Se–Ni–S angles negligibly to 89.3(4)° and the seven-membered ring of the xylenediyl group pushes the S1–Ni–S2 angle to 99.4(2)°. There is a small tetrahedral distortion from the square plane around the nickel center with an angle of 11.1 ± 1° between the planes Se1–Ni–S1 and Se2–Ni–S2. The corresponding tetrahedral distortion in [Ni('S₄')] is smaller at 4.31 ± 4°.^[17a]

Single crystals of [NiFe('S₂Se₂') (CO)₃] were grown from liquid diffusion of pentane into a saturated dichloromethane solution of the complex. The complex crystallizes in the space group *P*₂₁/*n* with a disordered pentane solvent molecule in half occupancy (Experimental Section and Supporting Information, Table S1). The X-ray crystal structure of [NiFe('S₂Se₂') (CO)₃] is shown in Figure 3B and it is structurally similar to the previously reported [NiFe('S₄') (CO)₃].^[15d] Selected bond distances and angles for [NiFe('S₂Se₂') (CO)₃] and [NiFe('S₄') (CO)₃]^[15d] are shown in Table 2. One of the 'S₂Se₂' selenolate donor ligands bridges the nickel and the iron centers, whereas the other remains terminally coordinated to the nickel center. One of the three carbonyl ligands takes up a bridging position between the nickel and the iron centers. One of the 'S₂Se₂' thioether donors has become uncoordinated from the nickel center and coordinates instead to iron whilst the other remains coordinated to nickel. The coordination geometry around the nickel center is distorted tetrahedral and around the iron center is square-based pyramidal.

[NiFe('S₂Se₂') (CO)₃] possesses a number of the key structural features of the [NiFeSe] hydrogenase active site including one nickel and one iron center held together by two bridging ligands and, most importantly, a selenolate donor terminally co-

Table 2. Selected bond distances and angles for [NiFe('S₂Se₂') (CO)₃] and [NiFe('S₄') (CO)₃]^[15d] model complexes.^[a]

[NiFe('S ₂ Se ₂ ') (CO) ₃] Distances [Å]	[NiFe('S ₄ ') (CO) ₃] Distances [Å]
Ni–Fe 2.4480(4)	Ni–Fe 2.4262(2)
Ni–Se1 2.2802(4)	Ni–S'1 2.1644(3)
Ni–S1 2.1940(6)	Ni–S1 2.1942(3)
Ni–Se2 2.2947(3)	Ni–S'2 2.1749(3)
Ni–C19 2.033(3)	Ni–C19 2.035(1)
Fe–Se2 2.3699(4)	Fe–S'2 2.2567(3)
Fe–C19 1.822(3)	Fe–C19 1.823 (1)
Fe–S2 2.3088(7)	Fe–S2 2.3058(4)
C19–O3 1.168(3)	C19–O3 1.165 (1)

[NiFe('S ₂ Se ₂ ') (CO) ₃] Angle [°]	[NiFe('S ₄ ') (CO) ₃] Angle [°]
Se1–Ni–S1 94.28 (2)	S'1–Ni–S1 93.38(4)
Se1–Ni–Se2 97.37 (1)	S'1–Ni–S'2 104.60(4)
Se1–Ni–C19 142.77(8)	S'1–Ni–C19 135.5(1)
Se1–Ni–Fe 156.40(1)	S'1–Ni–Fe 162.50(4)
C19–Fe–Ni 54.51(8)	C19–Fe–Ni 67.5(1)
C19–Ni–Fe 46.85(8)	C19–Ni–Fe 43.3(1)
Ni–Se2–Fe 63.29 (1)	Ni–S'2–Fe 67.25(3)

[a] S = thioether, S' = thiolate donor.

ordinated to the nickel center. All metal–selenium bonds in [NiFe('S₂Se₂') (CO)₃] are approximately 0.1 Å longer than the equivalent metal–sulfur bonds in [NiFe('S₄') (CO)₃].^[15d]

The distance between the nickel and the terminal selenium donor in [NiFe('S₂Se₂') (CO)₃] is 2.28 Å and the nickel selenolate bond distance was reported as 2.46 Å in the [NiFeSe] hydrogenase from *Desulfomicrobium baculatum* at 2.15 Å resolution.^[5] The Ni–Se distance in the structure of the active *Desulfomicrobium baculatum* [NiFeSe] hydrogenase is 0.25 Å longer than the equivalent Ni–S distance in the active *Desulfovibrio vulgaris* Miyazaki F [NiFe] hydrogenase structure, although a significant error can be expected in the enzyme structure owing to a high temperature factor for the Sec residue.^[5,19] Theoretical modeling studies show the Ni–Se distance to be 0.11 Å longer than the equivalent Ni–S distance in the [NiFe] hydrogenase, which correlates well with the bond distances observed in the small molecule models studied herein.^[20] The introduction of selenium does not significantly affect any of the other distances in the model complexes. The Ni–Fe distance is only marginally longer (0.02 Å) in [NiFe('S₂Se₂') (CO)₃] than in [NiFe('S₄') (CO)₃] and the distances between the metal, and the bridging carbonyl carbon are almost identical in both complexes. Theoretical modeling also showed that the introduction of selenium into the hydrogenase active site would not affect any other bond distances.^[20] The bond angles in the structure of [NiFe('S₂Se₂') (CO)₃] are similar to those in [NiFe('S₄') (CO)₃]. The angle between the planes of Ni–C19–Fe (Figure 3B) and Ni–Se2–Fe is 71.1(1)° and the equivalent angle in [NiFe('S₄') (CO)₃] is 69.7°.^[15d]

The diamagnetism of [NiFe('S₂Se₂') (CO)₃] as well as the short nickel–iron distance indicate that there is a bond between the two metal centers, which is supported by previously reported density functional theory studies of [NiFe('S₄') (CO)₃].^[15d]

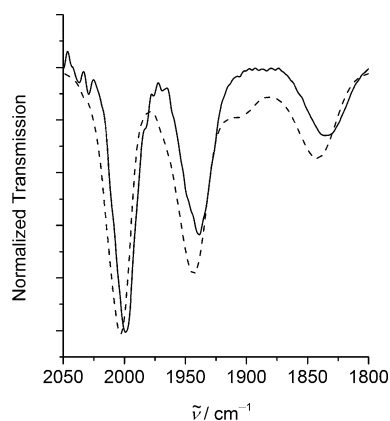


Figure 4. Attenuated total reflectance FTIR spectra of $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ (—) and $[\text{NiFe}(\text{S}_4')(\text{CO})_3]$ (- - -).

Spectroscopic characterization

Figure 4 shows the carbonyl region of the IR spectrum of $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ and $[\text{NiFe}(\text{S}_4')(\text{CO})_3]$. Complex $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ displays three carbonyl stretching bands: two at $\tilde{\nu} = 2000$ and 1942 cm^{-1} attributable to the terminally coordinated carbonyls and one at $\tilde{\nu} = 1835 \text{ cm}^{-1}$ for the bridging carbonyl. All three CO stretching frequencies for $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ are $1\text{--}7 \text{ cm}^{-1}$ lower than those for $[\text{NiFe}(\text{S}_4')(\text{CO})_3]$ ($\tilde{\nu} = 2003, 1943, \text{ and } 1842 \text{ cm}^{-1}$),^[15d] indicating increased π back-donation from the metal d orbitals to the carbonyl π^* antibonding orbital, owing to increased electron density at the metal centers from selenium. The same trend is observed in the $[\text{NiFeSe}]$ hydrogenase active sites. The stretching frequencies of the CO ligand terminally coordinated to iron in the Ni–C states of the $[\text{NiFeSe}]$ hydrogenase from *Desulfovibrio vulgaris* Miyazaki F ($\tilde{\nu} = 1948$ and 1925 cm^{-1} , two different isoforms)^[7g] and *Desulfovibrio vulgaris* Hildenborough ($\tilde{\nu} = 1915$ and 1900 cm^{-1} , two different isoforms)^[7f] are lower than those observed for the $[\text{NiFe}]$ hydrogenases ($\tilde{\nu} = 1961\text{--}1949 \text{ cm}^{-1}$).^[21] Furthermore, a CO inhibited *Desulfovibrio vulgaris* Hildenborough $[\text{NiFeSe}]$ hydrogenase, in which there is a CO ligand coordinated to the active site nickel center, exhibits a $5\text{--}15 \text{ cm}^{-1}$ shift to lower frequencies ($\tilde{\nu} = 2052, 2042 \text{ cm}^{-1}$, two different isoforms) compared to CO inhibited conventional $[\text{NiFe}]$ hydrogenases from *Desulfovibrio fructosovorans*, *Desulfovibrio vulgaris* Miyazaki F, and *Chromatium vinosum*.^[7f, 11e, 21b, 22]

The electronic absorption spectra of $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ and $[\text{NiFe}(\text{S}_4')(\text{CO})_3]$ in DMF are shown in Figure 5, and the corresponding data are given in the Supporting Information, Table S2. There are several strong bands in the visible region of the spectrum of $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$. The absorption bands exhibited by $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ are all red-shifted relative to $[\text{NiFe}(\text{S}_4')(\text{CO})_3]$, as expected due to the increased size and polarizability of selenium relative to sulfur.

Electrochemical characterization

The cyclic voltammograms of $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ and $[\text{NiFe}(\text{S}_4')(\text{CO})_3]$ (1 mM) exhibit two irreversible reduction waves in

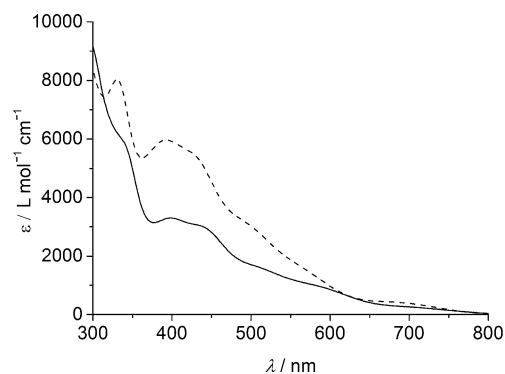


Figure 5. Electronic absorption spectra of $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ (—) and $[\text{NiFe}(\text{S}_4')(\text{CO})_3]$ (- - -) in DMF.

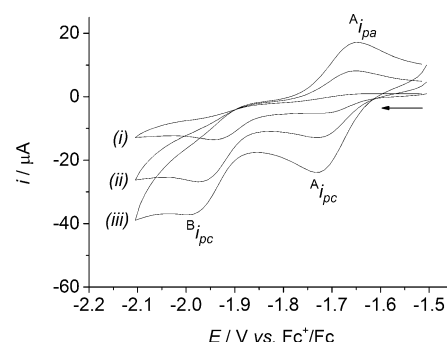


Figure 6. Cyclic voltammograms of $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ (1 mM) in acetonitrile (0.1 M *n*-Bu₄NBF₄) at a scan rate of *i*) 100, *ii*) 500, and *iii*) 1000 mV s^{-1} .

an acetonitrile electrolyte solution at 100 mV s^{-1} (Figure 6; Supporting Information, Figure S17). The first reduction wave, wave A, at $E_{\text{pc}} = -1.72$ and -1.76 V is followed by a second wave, wave B, at -1.98 V and -1.99 V vs. Fc^+/Fc for $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ and $[\text{NiFe}(\text{S}_4')(\text{CO})_3]$, respectively. The first reduction wave becomes almost reversible in both complexes at higher scan rates, with a peak current ratio ($A_{\text{ip}}/A_{\text{pc}}$) of approximately 0.8 at 1000 mV s^{-1} . In DMF electrolyte solution and at a scan rate of 100 mV s^{-1} , the two reduction waves are observed at $E_{\text{pc}} = -1.72$ and -1.98 V for $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ and at $E_{\text{pc}} = -1.79$ and -2.01 V vs. Fc^+/Fc for $[\text{NiFe}(\text{S}_4')(\text{CO})_3]$. Both the first and second reduction waves of $[\text{NiFe}(\text{S}_2\text{Se}_2')(\text{CO})_3]$ are shifted to more anodic potentials compared to $[\text{NiFe}(\text{S}_4')(\text{CO})_3]$, which is consistent with the substitution of a thiolate by a selenolate donor ligand in a number of other transition-metal complexes.^[15e,g,i-1,23] For example, this trend was observed with $[\text{FeFe}]$ hydrogenase model complexes with selenolate containing bridging ligands compared to the same complex with thiolate containing bridging ligands.^[15e,g,i-1]

The same trend is also exhibited by complexes $[\text{Ni}(\text{S}_2\text{Se}_2')]$ and $[\text{Ni}(\text{S}_4')]$, which exhibit one irreversible reduction wave in DMF at $E_{\text{pc}} = -1.87 \text{ V}$ and -1.99 V vs. Fc^+/Fc at 100 mV s^{-1} , respectively (Supporting Information, Figure S18). This reduction wave was previously assigned for $[\text{Ni}(\text{S}_4')]$ as the reduction of the nickel center.^[17c]

$[\text{NiFe}(\text{S}_4')(\text{CO})_3]$ was previously reported to behave as an electrocatalyst for H₂ production in an acetonitrile electrolyte

solution with trifluoroacetic acid (TFA).^[15d] We have repeated these experiments and also observed a catalytic wave in the cyclic voltammogram of $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$ in an acetonitrile electrolyte solution in the presence of TFA using a glassy carbon working electrode. The peak current of this catalytic wave increases with increasing concentrations of the acid. However, a control experiment showed that a comparable catalytic response was also observed when cyclic voltammograms were recorded on a bare glassy carbon electrode under the same conditions but in the absence of $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$. The linear sweep voltammograms on a glassy carbon electrode in the presence and absence of $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$ with increasing concentrations of TFA in acetonitrile are shown in the Supporting Information, Figure S19. Thus, $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$ does not display significant catalytic activity under these conditions (see further discussion below).

The catalytic activity of the complexes was thus assessed using DMF as a solvent as the pK_a of many organic acids in DMF is significantly higher than in acetonitrile.^[24] No catalytic current enhancement was detected using $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ or $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$ in DMF in the presence of acetic acid or benzoic acid, but with the stronger TFA, H_2 production activity was observed (Supporting Information, Figure S20). A catalytic wave appeared, which showed an increase in current with increasing acid concentrations, whereas voltammograms on a bare glassy carbon electrode under the same conditions with no complex gave negligible current enhancement (Supporting Information, Figure S21).

However, the catalytic response does not result from homogeneous catalysis, but a solid deposit on the electrode surface formed by the electrodeposition of either $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ or $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$. Following cyclic voltammetry of the complex in DMF with increasing concentrations of TFA (up to 100 mM; Supporting Information, Figure S20) the working electrode was removed from the solution and rinsed with DMF. This electrode was then placed in a fresh electrolyte solution (rinse test) containing 100 mM of TFA without any NiFe complex in solution and the same catalytic response was observed as with the complex in solution (Figure 7; Supporting Information, Figure S22). The stability of the complexes in TFA/DMF solution in the absence of an applied potential was established using electronic absorption spectroscopy, confirming that a solid deposit is formed through electrodeposition (Supporting Information, Figure S23).

A comparable catalyst precursor activity was also observed for our previously reported mononuclear nickel thiolate/selenolate complexes.^[18] Deposition of a growing number of first-row transition-metal complexes onto electrodes is being reported and the nature of the precursor complex affects the morphology and activity of the resulting heterogeneous catalyst.^[2d, 18, 25]

Thus, the composition of the deposit from $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ and $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$ was characterized to determine the nature of the catalytic species. A glassy carbon slide with a surface area of 1.6 cm^2 was modified with the deposit through electrodeposition from a solution of $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ or $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$ (1 mM) in the presence of

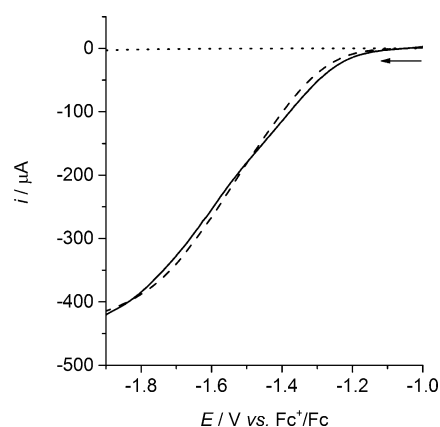


Figure 7. Linear sweep voltammograms of the solid deposit formed from electrodeposition of $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ in the presence (—) and absence (---) of dissolved $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ (1 mM) on a glassy carbon working electrode in DMF (0.1 M $n\text{-Bu}_4\text{NBF}_4$) containing TFA (100 mM) at a scan rate of 100 mV s^{-1} . The response of an unmodified (bare) glassy carbon working electrode in TFA/DMF (.....) is also shown (recorded in the absence of $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ in solution).

TFA (10 mM) in DMF at $E_{\text{appl}} = -1.75 \text{ V vs. Fc}^+/\text{Fc}$ for 0.5 h. The modified electrode was then removed from the solution and rinsed with DMF (3 mL) before analysis. Scanning electron microscopy (SEM) analysis of the slides treated with either $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ or $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$ revealed that in both cases the electrode is entirely covered in a film of the deposit (Figure 8). Energy-dispersive X-ray spectroscopy (EDX) analysis confirmed that both films consist mainly of nickel and iron (Figure 8; Supporting Information, Table S3). There is sulfur (4 atom%) and selenium (16 atom%) in the film deposited from $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ and sulfur (13 atom%) in the film deposited from $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$. Low levels of sulfur and selenium rule out the possibility that the bulk of the film material is a metal sulfide or metal selenide. Surface analysis by X-ray photoelectron spectroscopy (XPS) confirmed that the film sur-

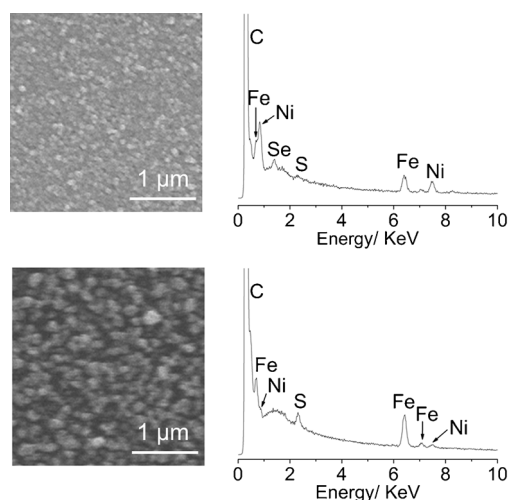


Figure 8. SEM and EDX analysis of films electrodeposited on a glassy carbon electrode from complexes $[\text{NiFe}(\text{S}_2\text{Se}_2)(\text{CO})_3]$ (top) and $[\text{NiFe}(\text{S}_4)(\text{CO})_3]$ (bottom).

face is mostly comprised of nickel and iron, with small amounts of sulfur and/or selenium (Supporting Information, Table S3). The Ni 2p signals in the XPS spectra of both deposits at 874 and 856 eV with satellites at 880 and 862 eV correspond to Ni(OH)₂ (Supporting Information, Figure S24). The surfaces were exposed to air before analysis so it is reasonable to assume that significant surface oxidation occurred. A small nickel(0) peak is visible in the Ni 2p spectrum of the deposit from [NiFe('S₂Se₂') (CO)₃] at 852 eV, which is possibly the catalytically active species. The Fe 2p signals in both deposits at 711 and 724 eV with satellites at 719 and 732 eV show that it is in the form of iron oxide and no resolvable iron(0) signal is observed.

The deposits from [NiFe('S₂Se₂') (CO)₃] and [NiFe('S₄') (CO)₃] on a glassy carbon disk or fluorine-doped tin oxide (FTO) electrode (electrodeposited at -1.75 V vs. Fc⁺/Fc for 0.5 h in a 1 mM solution of the complex in DMF containing 10 mM TFA) were also shown to be electroactive for H₂ evolution in an aqueous pH neutral phosphate solution (Supporting Information, Figure S25). The deposits show comparable activity to other nickel containing H₂ production catalyst films formed from molecular precursors recently reported.^[18,25d] Controlled potential electrolysis of such films on an FTO electrode with a surface area of 1.6 cm² confirmed the generation of H₂ (head-space gas chromatography analysis).

Conclusion

A synthetic structural model of the [NiFeSe] hydrogenase active site has been reported. The complex was synthesized using the nickel precursor complex [Ni('S₂Se₂')], in which the nickel center is surrounded by two selenolate and two thioether donors. Complex [NiFe('S₂Se₂') (CO)₃] mimics several of the main structural features of the enzyme active site, including one nickel and one iron center held together by two bridging ligands and a selenolate donor terminally coordinated to the nickel center. Relevant distances and angles in [NiFe('S₂Se₂') (CO)₃] agree well with those found in the enzyme. The nickel-selenium distance is 0.1 Å longer and the nickel-iron distance in [NiFe('S₂Se₂') (CO)₃] only slightly longer than the analogous [NiFe] hydrogenase model complex [NiFe('S₄') (CO)₃]. The metal-carbonyl bond lengths in the two complexes are almost identical. The differences in the spectroscopic properties of [NiFe('S₂Se₂') (CO)₃] and [NiFe('S₄') (CO)₃] illustrate the differences in their electronic structures. IR spectroscopy revealed that the carbonyl bands in [NiFe('S₂Se₂') (CO)₃] are all shifted to lower frequencies relative to [NiFe('S₄') (CO)₃], indicating that the more electron-donating selenolate groups offer an increased electron density at the Fe center. The signals in the electronic absorption spectrum of [NiFe('S₂Se₂') (CO)₃] are shifted to lower energies than in [NiFe('S₄') (CO)₃]. Extensive electrochemical studies revealed that both NiFe complexes do not behave as homogenous catalysts for H₂ evolution, but are molecular precursors for active heterogeneous catalysts, which can be readily electrodeposited onto an electrode surface. Analysis of the solid deposits shows that these films contain nickel and iron with some sulfur and selenium. The deposit is

electrocatalytically active for proton reduction in organic solvents with acid or aqueous pH neutral phosphate solution.

Experimental Section

Materials and methods

All of the complexes were synthesized using anhydrous anaerobic techniques using a Schlenk line unless otherwise noted. All starting materials were purchased from commercial suppliers in the highest available purity for all analytical measurements and used without further purification. Organic solvents were dried and deoxygenated prior to use. Bis(3-chloro-2,2-methyl-1-thiapropyl)-*o*-xylene and [Ni('S₄')],^[17a] [Fe(CO)₃bda],^[26] and [NiFe('S₄') (CO)₃]^[15d] have been synthesized using previously reported procedures. Electrochemistry-grade *n*-Bu₄NBF₄ electrolyte was purchased from Sigma Aldrich. The glassy carbon electrodes were cleaned by first cycling at positive potentials in 1 M hydrochloric acid using a silver wire pseudo reference electrode and then polishing using alumina powder (1 μm diameter).

Physical measurements

NMR spectra were recorded on a Bruker DPX-400 MHz spectrometer and the spectra were referenced against the solvent peak. The mass spectrum of 'S₂Se₂'_{pre} was recorded by the University of Cambridge Mass Spectrometry Service using a Bruker Bio Apex 4.0 FTICR ESI-MS. The mass spectra of the metal complexes were recorded on a Waters Quattro LC electrospray ionization mass spectrometer. Expected and experimental isotope distributions of the compounds were compared. Elemental analysis was carried out by the microanalysis service of the Department of Chemistry, University of Cambridge. FTIR spectra were recorded on a ThermoScientific Nicolet i550 FTIR spectrometer with an ATR sampling accessory. Electronic absorption spectra were recorded on an Agilent Cary UV-Vis 50 Bio spectrometer. The SEM images and EDX spectra were recorded using a Philips XL30 132-10 electron microscope. EDX studies (edax PV7760/68 ME) were run at a 15 kV acceleration voltage, spot size 4.0, and an acquisition time of at least 100 s. The elements were assigned and atomic ratios were identified using the built-in software (EDAX). XPS data were obtained at the National EPSRC XPS User's Service (NEXUS) at Newcastle University, UK, an EPSRC Mid-range Facility. Analysis was performed using a Kα spectrometer (Thermo Scientific, East Grinstead, UK) utilizing a monochromatic AlKα X-ray source (1486.6 eV, 400 μm spot size, 36 W).

X-ray crystallographic studies

Data were recorded with MoKα radiation ($\lambda = 0.71073$ Å) on a Nonius Kappa CCD diffractometer fitted with an Oxford Cryosystems Cryostream cooling apparatus. The single crystal was mounted in Paratone N oil on the tip of a glass fiber and kept under a stream of N₂. Structure solution was carried out using direct methods and refined by least squares (SHELXL-97)^[27] using Chebyshev weights on F_o^2 . The weighted *R*-factor *wR* and goodness of fit (GOF) are based on F^2 . Crystal data, data collection parameters, and structure refinement details for the complexes are given in the Supporting Information, Table S1. The structure of complex [Ni('S₂Se₂')] contained two crystallographically independent molecules in the asymmetric unit. A poorly resolved pentane solvent molecule co-crystallized with [NiFe('S₂Se₂') (CO)₃] and it was modeled as one half-weight molecule disordered about an inversion center with geometric restraints and a common isotropic displace-

ment parameter for the carbon atoms. Selected bond distances and angles are shown in Tables 1 and 2. The mean bond distances and angles for the discussion in the paper were calculated as follows: for a sample of n observations x_i , a weighted mean value (x_w) with its standard deviation (σ) was calculated using the following equations: $x_w = \sum x_i/n$, $\sigma = \{\sum (x_i - x_w)^2 / [n(n-1)]\}^{1/2}$. Crystal structure images were created using Ortep 3 for Windows.^[28] CCDC 1050563 ([Ni('S₂Se₂')]) and CCDC 1050564 ([NiFe('S₂Se₂')(3] \cdot 0.5 C₃H₁₂]) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Electrochemical measurements

Voltammograms were recorded at room temperature under inert gas using an IviumStat or CompactStat potentiostat. A standard three-electrode cell was used for all measurements with a glassy carbon disk working (3 mm diameter), a platinum mesh counter, and a Ag/Ag⁺ (organic solutions) or Ag/AgCl/KCl_(sat) (aqueous solutions) reference electrode. For voltammograms recorded in organic solvents containing *n*-Bu₄NBF₄ (0.1 M), the Fc⁺/Fc couple was used as a reference. For voltammograms recorded in a pH 7 aqueous phosphate solution (0.1 M), potentials were converted to the normal hydrogen electrode (NHE) by adding 0.2 V to the potential against Ag/AgCl/KCl_(sat).^[29] Unless otherwise stated, the second of consecutive scans is shown, as currents were diffusion limited on this scan and all subsequent scans were identical.

For deposition of the films for characterization, a glassy carbon slide (1 cm x 1 cm x 0.1 cm) was immersed in a solution of [NiFe('S₂Se₂')(3] or [NiFe('S₄')(3] (1 mM) in the presence of TFA (10 mM) in DMF with *n*-Bu₄NBF₄ (0.1 M). An electrode surface area of 1.6 cm² was in contact with the electrolyte solution. A potential of approximately -1.75 V vs. Fc⁺/Fc was applied for 0.5 h. The modified electrode was then removed from the solution and rinsed with DMF (3 mL).

Catalytic films for controlled potential electrolysis were deposited from a solution of [NiFe('S₂Se₂')(3] or [NiFe('S₄')(3] (1 mM) in the presence of TFA (10 mM) in DMF with *n*-Bu₄NBF₄ (0.1 M) on a glassy carbon or FTO-coated glass electrode (geometric surface area in contact with electrolyte solution of approximately 1.6 cm²) at -1.75 V vs. Fc⁺/Fc for 0.5 h. The modified electrode was then removed from the solution, rinsed with DMF (3 mL) and immersed into an aqueous phosphate solution (0.1 M, pH 7). Controlled potential electrolysis was carried out in an airtight electrochemical cell containing N₂ with 2% methane as internal standard for gas chromatography (GC) analysis. The headspace gas was analyzed using an Agilent 7890 A GC equipped with a 5 Å molecular sieve column, using N₂ carrier gas with a flow rate of approximately 3 mL min⁻¹. The GC columns were kept at 40 °C and a thermal conductivity detector was used.

Synthesis and characterization

Synthesis of 'S₂Se₂'_{pre}: A solution of selenourea (1.40 g, 11.4 mmol) in ethanol (25 mL) was added to a solution of bis(3-chloro-2,2-methyl-1-thiopropyl)-*o*-xylene (2.00 g, 5.7 mmol) in ethanol (10 mL) and the colorless solution was refluxed for 30 min, during which time a white solid precipitated. The reaction mixture was cooled on ice, the solid product was isolated by filtration, washed with cold ethanol (3 x 5 mL) and diethyl ether (3 x 5 mL), and dried under high vacuum at room temperature. Yield: 1.91 g, 61%. ¹H NMR (400 MHz, D₂O): δ = 7.45 (2H, m, Ar), 7.35 (2H, m, Ar), 4.07 (4H, s, CH₂), 3.62 (4H, s, CH₂), 1.57 (12H, s, CH₃); ¹³C NMR (400 MHz, D₂O): δ = 168.15 (C(NH)NH₂), 135.34 (Ar), 131.00 (Ar),

128.37 (Ar), 46.54 (CMe₂), 41.31 (CH₂), 30.17 (CH₂), 27.61 ppm (Me); ATR-IR: $\tilde{\nu}$ = 3011, 2960, 1630, 1416, 693 cm⁻¹; ESI-MS (H₂O) + ve: 527 (100%, C₁₈H₃₁N₄S₂Se₂⁺); elemental analysis calcd (%) for C₁₈H₃₂Cl₂N₄S₂Se₂: C 36.19, H 5.40, Cl 11.87, N 9.38; found: C 36.28, H 5.30, Cl 11.97, N 9.08.

Synthesis of [Ni('S₂Se₂')]: A solution of NMe₄OH \cdot 5H₂O (121 mg, 668 μ mol) in ethanol (4 mL) was added to a suspension of 'S₂Se₂'_{pre} (200 mg, 335 μ mol) in ethanol (4 mL) and the reaction mixture was stirred for 10 min until the white solid had dissolved. The solution was then added to a suspension of [Ni(acac)₂] (86 mg, 335 μ mol) in ethanol (20 mL), and the reaction mixture was heated to reflux for 1 h, during which time a green solution had formed. The solvent volume was reduced to 10 mL and the resulting green precipitate was separated by filtration, washed with ethanol (3 x 3 mL), and dried under vacuum. The product was recrystallized by slow liquid diffusion of hexane into a dichloromethane solution of the complex. Yield: 138 mg, 83%. ¹H NMR (400 MHz, CD₂Cl₂): δ = 7.33 (2H, m, Ar), 7.28 (2H, m, Ar), 3.90 (4H, s, CH₂), 2.36 (4H, s, CH₂), 1.73 ppm (12H, s, CH₃); ¹³C NMR (400 MHz, CD₂Cl₂): δ = 134.43 (Ar), 131.32 (Ar), 129.70 (Ar), 65.67, 33.68, 28.34, 26.90 ppm; ESI-MS (CHCl₃) + ve: 498 (100%, [M]⁺); elemental analysis calcd (%) for C₁₆H₂₄NiS₂Se₂: C 38.66, H 4.87; found: C 38.67, H 4.68; λ_{\max} (CH₂Cl₂) = 290 nm (ϵ = 17.2 x 10³ L mol⁻¹ cm⁻¹). Single crystals for X-ray analysis were grown from liquid diffusion of hexane into a dichloromethane solution of the complex.

Synthesis of [NiFe('S₂Se₂')(3]: A solution of [Fe(CO)₃(bda)] (50 mg, 174 μ mol) in dichloromethane (3 mL) was added to a solution of [Ni('S₂Se₂')] (86 mg, 174 μ mol) in dichloromethane (3 mL) and the resulting red solution was stirred at room temperature. The reaction was followed by monitoring the disappearance of the carbonyl-stretches in [Fe(CO)₃(bda)] at 2069, 2007, and 1988 cm⁻¹ by ATR-IR spectroscopy. The reaction was complete after approximately 1 h, whereupon the solvent was removed under high vacuum and the product was purified using column chromatography (SiO₂, dichloromethane) to give a red solid. Yield: 48 mg, 43%. ¹H NMR (400 MHz, CD₂Cl₂): δ = 7.52 (1H, m, Ar), 7.36 (3H, m, Ar), 4.84 (1H, d, CH₂), 4.24 (1H, d, CH₂), 3.97 (1H, d, CH₂), 3.82 (1H, d, CH₂), 3.10 (1H, d, CH₂), 3.01 (1H, d, CH₂), 2.80 (1H, d, CH₂), 2.72 (1H, d, CH₂), 1.87 (3H, s, CH₃), 1.85 (3H, s, CH₃), 1.63 (3H, s, CH₃), 1.41 ppm (3H, s, CH₃); ¹³C NMR (400 MHz, CD₂Cl₂): δ = 219.12 (CO), 134.33 (Ar), 133.68 (Ar), 132.65 (Ar), 131.40 (Ar), 129.19 (Ar), 129.06 (Ar), 66.16 (CMe₂), 61.71 (CMe₂), 39.98 (CH₂), 35.88 (CH₂), 34.66 (CH₂), 29.18 (CH₂), 27.98 (Me), 27.57 (Me), 25.95 (Me), 25.52 ppm (Me); ATR-IR: $\tilde{\nu}$ = 2000 (CO), 1942 (CO), 1835 cm⁻¹ (CO); ESI-MS (CH₂Cl₂) + ve: 638 (100% [M]⁺); elemental analysis calcd (%) for C₁₉H₂₄FeNiO₃S₂Se₂: C 35.83, H 3.80; found: C 36.53, H 3.81; λ_{\max} (DMF) = 399 nm (ϵ = 5.79 x 10³ L mol⁻¹ cm⁻¹). Single crystals were grown from liquid diffusion of pentane into a dichloromethane solution of the complex.

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Keywords: active sites • enzyme models • hydrogenase • selenium • structural models

- [1] a) J. A. Turner, *Science* **2004**, *305*, 972–974; b) H. B. Gray, *Nat. Chem.* **2009**, *1*, 7; c) B. E. Barton, T. B. Rauchfuss, *J. Am. Chem. Soc.* **2010**, *132*, 14877–14885; d) C.-Y. Lin, D. Mersch, D. Jefferson, E. Reisner, *Chem. Sci.* **2014**, *5*, 4906–4913.
- [2] a) Y. Shim, R. M. Young, A. P. Douvalis, S. M. Dyar, B. D. Yuhas, T. Bakas, M. R. Wasielewski, M. G. Kanatzidis, *J. Am. Chem. Soc.* **2014**, *136*, 13371–13380; b) M. S. Faber, R. Dziejczak, M. A. Lukowski, N. S. Kaiser, Q. Ding, S. Jin, *J. Am. Chem. Soc.* **2014**, *136*, 10053–10061; c) J. Kibsgaard, T. F. Jaramillo, F. Besenbacher, *Nat. Chem.* **2014**, *6*, 248–253; d) Y.-H. Lai, H. S. Park, J. Z. Zhang, P. D. Matthews, D. S. Wright, E. Reisner, *Chem. Eur. J.* **2015**, *21*, 3919–3923.
- [3] a) K. A. Vincent, A. Parkin, F. A. Armstrong, *Chem. Rev.* **2007**, *107*, 4366–4413; b) F. A. Armstrong, N. A. Belsey, J. A. Cracknell, G. Goldet, A. Parkin, E. Reisner, K. A. Vincent, A. F. Wait, *Chem. Soc. Rev.* **2009**, *38*, 36–51; c) E. Reisner, *Eur. J. Inorg. Chem.* **2011**, 1005–1016.
- [4] W. Lubitz, H. Ogata, O. Rüdiger, E. Reijerse, *Chem. Rev.* **2014**, *114*, 4081–4148.
- [5] E. Garcin, X. Vernede, E. C. Hatchikian, A. Volbeda, M. Frey, J. C. Fontecilla-Camps, *Structure* **1999**, *7*, 557–566.
- [6] C. S. A. Baltazar, M. C. Marques, C. M. Soares, A. M. De Lacey, I. A. C. Pereira, P. M. Matias, *Eur. J. Inorg. Chem.* **2011**, 948–962.
- [7] a) P. M. Vignais, L. Cournac, E. C. Hatchikian, S. Elsen, L. Serebryakova, N. Zorin, B. Dimon, *Int. J. Hydrogen Energy* **2002**, *27*, 1441–1448; b) F. M. A. Valente, A. S. F. Oliveira, N. Gnadt, I. Pacheco, A. V. Coelho, A. V. Xavier, M. Teixeira, C. M. Soares, I. A. C. Pereira, *J. Biol. Inorg. Chem.* **2005**, *10*, 667–682; c) F. M. A. Valente, C. C. Almeida, I. Pacheco, J. Carita, L. M. Saraiva, I. A. C. Pereira, *J. Bacteriol.* **2006**, *188*, 3228–3235; d) A. Parkin, G. Goldet, C. Cavazza, J. C. Fontecilla-Camps, F. A. Armstrong, *J. Am. Chem. Soc.* **2008**, *130*, 13410–13416; e) E. Reisner, D. J. Powell, C. Cavazza, J. C. Fontecilla-Camps, F. A. Armstrong, *J. Am. Chem. Soc.* **2009**, *131*, 18457–18466; f) C. Gutiérrez-Sánchez, O. Rüdiger, V. M. Fernández, A. L. De Lacey, M. Marques, I. A. C. Pereira, *J. Biol. Inorg. Chem.* **2010**, *15*, 1285–1292; g) J. Riethausen, O. Rüdiger, W. Gärtner, W. Lubitz, H. S. Shafaat, *ChemBioChem* **2013**, *14*, 1714–1719; h) K. Nonaka, N. T. Nguyen, K.-S. Yoon, S. Ogo, *J. Biosci. Bioeng.* **2013**, *115*, 366–371.
- [8] a) T. Sakai, D. Mersch, E. Reisner, *Angew. Chem. Int. Ed.* **2013**, *52*, 12313–12316; *Angew. Chem.* **2013**, *125*, 12539–12542; b) C. A. Caputo, M. A. Gross, V. W. Lau, C. Cavazza, B. V. Lotsch, E. Reisner, *Angew. Chem. Int. Ed.* **2014**, *53*, 11538–11542; *Angew. Chem.* **2014**, *126*, 11722–11726.
- [9] a) E. S. J. Arnér, *Exp. Cell Res.* **2010**, *316*, 1296–1303; b) A. Bassegoda, C. Madden, D. W. Wakerley, E. Reisner, J. Hirst, *J. Am. Chem. Soc.* **2014**, *136*, 15473–15476.
- [10] a) P. M. Matias, C. M. Soares, L. M. Saraiva, R. Coelho, J. Morais, J. Le Gall, M. A. Carrondo, *J. Biol. Inorg. Chem.* **2001**, *6*, 63–81; b) A. Volbeda, Y. Montet, X. Vernède, E. C. Hatchikian, J. C. Fontecilla-Camps, *Int. J. Hydrogen Energy* **2002**, *27*, 1449–1461.
- [11] a) A. Volbeda, M.-H. Charon, C. Piras, E. C. Hatchikian, M. Frey, J. C. Fontecilla-Camps, *Nature* **1995**, *373*, 580–587; b) H. Ogata, S. Hirota, A. Nakahara, H. Komori, N. Shibata, T. Kato, K. Kano, Y. Higuchi, *Structure* **2005**, *13*, 1635–1642; c) A. Volbeda, L. Martin, C. Cavazza, M. Matho, B. W. Faber, W. Roseboom, S. P. J. Albracht, E. Garcin, M. Rousset, J. C. Fontecilla-Camps, *J. Biol. Inorg. Chem.* **2005**, *10*, 239–249; d) M. van Gastel, C. Fichtner, F. Neese, W. Lubitz, *Biochem. Soc. Trans.* **2005**, *33*, 7–11; e) M.-E. Pandelia, H. Ogata, L. J. Currell, M. Flores, W. Lubitz, *Biochim. Biophys. Acta* **2010**, *1797*, 304–313.
- [12] a) M. Teixeira, G. Fauque, I. Moura, P. A. Lespinat, Y. Berlier, B. Prickril, H. D. Peck, Jr., A. V. Xavier, J. Le Gall, J. J. G. Moura, *Eur. J. Biochem.* **1987**, *167*, 47–58; b) M. Medina, E. C. Hatchikian, R. Cammack, *Biochim. Biophys. Acta* **1996**, *1275*, 227–236; c) M. C. Marques, R. Coelho, A. L. De Lacey, I. A. C. Pereira, P. M. Matias, *J. Mol. Biol.* **2010**, *396*, 893–907; d) M. C. Marques, R. Coelho, I. A. C. Pereira, P. M. Matias, *Int. J. Hydrogen Energy* **2013**, *38*, 8664–8682.
- [13] A. Volbeda, P. Amara, M. Iannello, A. L. De Lacey, C. Cavazza, J. C. Fontecilla-Camps, *Chem. Commun.* **2013**, 49, 7061–7063.
- [14] a) C. S. A. Baltazar, V. H. Teixeira, C. M. Soares, *J. Biol. Inorg. Chem.* **2012**, *17*, 543–555; b) A. Abou Hamdan, S. Dementin, P.-P. Liebmann, O. Gutiérrez-Sanz, P. Richaud, A. L. De Lacey, M. Rousset, P. Bertrand, L. Cournac, C. Léger, *J. Am. Chem. Soc.* **2012**, *134*, 8368–8371; c) O. Gutiérrez-Sanz, M. C. Marques, C. S. A. Baltazar, V. M. Fernandez, C. M. Soares, I. A. C. Pereira, A. L. De Lacey, *J. Biol. Inorg. Chem.* **2013**, *18*, 419–427.
- [15] a) D. Zheng, N. Wang, M. Wang, S. Ding, C. Ma, M. Y. Darensbourg, M. B. Hall, L. Sun, *J. Am. Chem. Soc.* **2014**, *136*, 16817–16823; b) A. Rahaman, S. Ghosh, D. G. Unwin, S. Basak-Modi, K. B. Holt, S. E. Kabir, E. Nordlander, M. G. Richmond, G. Hogarth, *Organometallics* **2014**, *33*, 1356–1366; c) E. P. Broering, P. T. Truong, E. M. Gale, T. C. Harrop, *Biochemistry* **2013**, *52*, 4–18; d) K. Weber, T. Krämer, H. S. Shafaat, T. Weyhermüller, E. Bill, M. van Gastel, F. Neese, W. Lubitz, *J. Am. Chem. Soc.* **2012**, *134*, 20745–20755; e) M. K. Harb, J. Windhager, T. Niksch, H. Görls, T. Sakamoto, E. R. Smith, R. S. Glass, D. L. Lichtenberger, D. H. Evans, M. El-khateeb, W. Weigand, *Tetrahedron* **2012**, *68*, 10592–10599; f) S. Canaguier, M. Field, Y. Oudart, J. Pécaut, M. Fontecave, V. Artero, *Chem. Commun.* **2010**, 46, 5876–5878; g) M. K. Harb, H. Görls, T. Sakamoto, G. A. N. Felton, D. H. Evans, R. S. Glass, D. L. Lichtenberger, M. El-khateeb, W. Weigand, *Eur. J. Inorg. Chem.* **2010**, 3976–3985; h) K. Ichikawa, T. Matsumoto, S. Ogo, *J. Chem. Soc. Dalton Trans.* **2009**, 4304–4309; i) M. K. Harb, U.-P. Apfel, J. Kübel, H. Görls, G. A. N. Felton, T. Sakamoto, D. H. Evans, R. S. Glass, D. L. Lichtenberger, M. El-khateeb, W. Weigand, *Organometallics* **2009**, *28*, 6666–6675; j) M. K. Harb, T. Niksch, J. Windhager, H. Görls, R. Holze, L. T. Lockett, N. Okumura, D. H. Evans, R. S. Glass, D. L. Lichtenberger, M. El-khateeb, W. Weigand, *Organometallics* **2009**, *28*, 1039–1048; k) U.-P. Apfel, Y. Halpin, M. Gottschaldt, H. Görls, J. G. Vos, W. Weigand, *Eur. J. Inorg. Chem.* **2008**, 5112–5118; l) S. Gao, J. Fan, S. Sun, X. Peng, X. Zhao, J. Hou, *J. Chem. Soc. Dalton Trans.* **2008**, 2128–2135; m) Z. Li, Y. Ohki, K. Tatsumi, *J. Am. Chem. Soc.* **2005**, *127*, 8950–8951; n) W. Zhu, A. C. Marr, Q. Wang, F. Neese, D. J. E. Spencer, A. J. Blake, P. A. Cooke, C. Wilson, M. Schröder, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 18280–18285.
- [16] a) C.-H. Lai, J. H. Reibenspies, M. Y. Darensbourg, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2390–2393; *Angew. Chem.* **1996**, *108*, 2551–2554; b) J. A. W. Verhagen, M. Lutz, A. L. Spek, E. Bouwman, *Eur. J. Inorg. Chem.* **2003**, 3968–3974; c) S. Ogo, K. Ichikawa, T. Kishima, T. Matsumoto, H. Nakai, K. Kusaka, T. Ohhara, *Science* **2013**, *339*, 682–684.
- [17] a) J. A. W. Verhagen, D. D. Ellis, M. Lutz, A. L. Spek, E. Bouwman, *J. Chem. Soc. Dalton Trans.* **2002**, 1275–1280; b) Y. Oudart, V. Artero, J. Pécaut, M. Fontecave, *Inorg. Chem.* **2006**, *45*, 4334–4336; c) Y. Oudart, V. Artero, J. Pécaut, C. Lebrun, M. Fontecave, *Eur. J. Inorg. Chem.* **2007**, 2613–2626; d) S. Canaguier, L. Vaccaro, V. Artero, R. Ostermann, J. Pécaut, M. J. Field, M. Fontecave, *Chem. Eur. J.* **2009**, *15*, 9350–9364; e) V. Fourmond, S. Canaguier, B. Golly, M. J. Field, M. Fontecave, V. Artero, *Energy Environ. Sci.* **2011**, *4*, 2417–2427; f) S. Canaguier, M. Fontecave, V. Artero, *Eur. J. Inorg. Chem.* **2011**, 1094–1099; g) S. Canaguier, V. Fourmond, C. U. Perotto, J. Fize, J. Pécaut, M. Fontecave, M. J. Field, V. Artero, *Chem. Commun.* **2013**, 49, 5004–5006.
- [18] C. Wombwell, E. Reisner, *Dalton Trans.* **2014**, 43, 4483–4493.
- [19] Y. Higuchi, H. Ogata, K. Miki, N. Yasuoka, T. Yagi, *Structure* **1999**, *7*, 549–556.
- [20] M. Stein, W. Lubitz, *Phys. Chem. Chem. Phys.* **2001**, *3*, 5115–5120.
- [21] a) A. Volbeda, E. Garcin, C. Piras, A. L. De Lacey, V. M. Fernandez, E. C. Hatchikian, M. Frey, J. C. Fontecilla-Camps, *J. Am. Chem. Soc.* **1996**, *118*, 12989–12996; b) A. L. DeLacey, C. Stadler, V. M. Fernandez, E. C. Hatchikian, H.-J. Fan, S. Li, M. B. Hall, *J. Biol. Inorg. Chem.* **2002**, *7*, 318–326; c) B. Bleijlevens, F. A. van Broekhuizen, A. L. De Lacey, W. Roseboom, V. M. Fernandez, S. P. J. Albracht, *J. Biol. Inorg. Chem.* **2004**, *9*, 743–752; d) C. Fichtner, C. Laurich, E. Bothe, W. Lubitz, *Biochemistry* **2006**, *45*, 9706–9716.
- [22] K. A. Bagley, C. J. Van Garderen, M. Chen, E. C. Duin, S. P. J. Albracht, W. H. Woodruff, *Biochemistry* **1994**, *33*, 9229–9236.
- [23] a) B. Kersting, *Eur. J. Inorg. Chem.* **1998**, 1071–1077; b) T. Kückmann, F. Schödel, I. Sängler, M. Bolte, M. Wagner, H.-W. Lerner, *Eur. J. Inorg. Chem.* **2010**, 468–475; c) B. Zheng, X.-D. Chen, S.-L. Zheng, R. H. Holm, *J. Am. Chem. Soc.* **2012**, *134*, 6479–6490; d) L.-C. Song, X.-J. Sun, G.-J. Jia, M.-M. Wang, H.-B. Song, *J. Organomet. Chem.* **2014**, *761*, 10–19.
- [24] V. Fourmond, P.-A. Jacques, M. Fontecave, V. Artero, *Inorg. Chem.* **2010**, *49*, 10338–10347.
- [25] a) E. Anxolabéhère-Mallart, C. Costentin, M. Fournier, S. Nowak, M. Robert, J.-M. Savéant, *J. Am. Chem. Soc.* **2012**, *134*, 6104–6107; b) M. Fang, M. H. Engelhard, Z. Zhu, M. L. Helm, J. A. S. Roberts, *ACS Catal.* **2014**, *4*, 90–98; c) Y.-H. Lai, C.-Y. Lin, Y. Lv, T. C. King, A. Steiner, N. M.

- Muresan, L. Gan, D. S. Wright, E. Reisner, *Chem. Commun.* **2013**, *49*, 4331–4333; d) S. Cherdo, S. El Ghachtouli, M. Sircoglou, F. Brisset, M. Orio, A. Aukauloo, *Chem. Commun.* **2014**, *50*, 13514–13516.
- [26] J. A. S. Howell, B. F. G. Johnson, P. L. Josty, J. Lewis, *J. Organomet. Chem.* **1972**, *39*, 329–333.
- [27] G. M. Sheldrick, SHELXL-97, Program for Crystal Structure Refinement, University Göttingen, Göttingen, Germany **1997**.
- [28] L. Farrugia, *J. Appl. Crystallogr.* **1997**, *30*, 565.
- [29] A. J. Bard, L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, Wiley, **2001**.

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