1 General Introduction

1.1 Iron in Biology

The element iron is essential for life and plays a key role in biology.[1] In organisms, a large proportion of enzymes and cofactors require iron for their activity.[2] Photosynthesis, respiration and nitrogen fixation are some examples of fundamental biological processes that are based on iron-containing metalloproteins.[2] Iron is the most abundant transition metal in humans, healthy adults possess approximately 4.5 g of this element.[3] The majority of iron is bound to hemoglobin and myoglobin, or is stored by ferritin and hemosiderin. Less than 1 % of the iron is used by enzymes and redox proteins or is being transported by transferrin.[1] Despite the ubiquitous distribution and abundance of iron in the biosphere, organisms must contend with the hazards of iron deficiency and iron overload, which both lead to life-threating consequences.[4]

Under ambient aerobic conditions, iron is usually present as highly insoluble ferric (Fe^{3+}) hydroxide.[5] For biological utilization organisms assimilate Fe^{3+} with siderophores (small chelating molecules), such as, for example, enterobactin.[5] Reduction to ferrous iron (Fe^{2+}) decreases the binding constant of siderophores and simplifies further transport and storage, as Fe^{2+} is significantly more soluble under physiological conditions.[5] However, one-electron oxidation of Fe^{2+} by dioxygen produces superoxide ($O_2^{\bullet-}$), which in turn leads to the hydroxyl radical ($HO^{\bullet}$) *via* the Fenton sequence.[4] The hydroxyl radical is one of the most powerful oxidants encountered in biological systems. It attacks proteins, nucleic acids as well as carbohydrates, and initiates chain-propagating lipid peroxidation.[4] Due to the poor bioavailability of ferric iron and the high toxicity of ferrous iron, evolution has developed homeostatic mechanisms to regulate the iron's mobilization, transport and storage.[4]

In organisms, the highest iron concentration exists in storage proteins like ferritin. This supramolecule consists of 24 spherically arranged subunits with a total capacity of up to 4500 ferric ions.[6] However, the great majority of iron-proteins contain only mono- or binuclear metal centers. In the case of iron-sulfur clusters, a maximum of eight iron atoms has been found accumulated (P^N-cluster in nitrogenase).[7] From a structural viewpoint iron-proteins can be divided into heme and non-heme proteins, depending on the ligand sphere of the metal. Iron-proteins with a heme scaffold such as peroxidases (peroxide oxidation), oxidoreductases (electron transfer), heme oxygenases (oxidations and oxygenations), hemoglobin and myoglobin (oxygen transport) contain a planar tetrapyrrole ligand and one or two axial ligands.[8] Non-heme proteins like iron-sulfur clusters (primarily electron transport), mono- and dioxygenases (oxidations and oxygenations), hemerythrin (oxygen transport), hydrogenases (hydrogen conversion), ferritin (iron storage) and transferrins (iron transport) are coordinated

by amino acids from surrounding peptides and often additional exogenous ligands.[8] The large number of iron-containing metalloproteins is again exceeded by the number of functions they perform.[8] For [NiFe] hydrogenases, [2Fe–2S] clusters, and oxygenases more detailed structural and functional descriptions are provided in the following chapters.

1.2 Why Iron?

According to the protein data base (PDB), 6 % of all proteins that have been elucidated by X-ray crystallography contain at least one iron atom; only proteins with magnesium and zinc sites are more common in nature.[9] However, demanding thermodynamic redox transformations (*e.g.* water oxidation, respiration, dinitrogen splitting, hydrogen conversion, C–H bond activation) are usually catalyzed by enzymes with transition metal cofactors such as Fe, Mn, Mo, V, Co, Cu and Ni. With respect to the large number of iron-dependent enzymes and cofactors that are listed in the introduction one may raise the question: Why did nature predominantly choose iron from all transition metals?

An obvious point is the natural abundance of iron in the Earth's crust (5 wt%), which is more prevalent than any other transition metal and only exceeded by the elements O, Si and Al.[10,11] On the other hand, the major share of iron is bound in minerals.[5] In seawater the concentration of iron is approximately 0.001 μM, thus the bioavailability is in fact rather poor compared to Mo (0.1 μM) or alkaline earth metals like Mg (50 mM) or Zn (0.01 μM).[5] The origin of most iron-containing cofactors presumably dates back to the early stages of evolution when bioavailability of iron was much higher under an anoxic atmosphere.[12] At that time soluble ferrous iron was ubiquitous. It has even been suggested that the precambrian oceanic photosynthesis by cyanobacteria may have been fueled by Fe^{2+} as an electron source, thereby leading to an oxic atmosphere and a decrease of the iron concentration in seawater.[13] However, there have to be intrinsic advantages of iron, otherwise it would have been replaced with another element by evolution after the change to an oxic atmosphere.

The catalytic activity of redox-active cofactors strongly depends on the activation energy which is in turn defined by the redox potential and the reorganization energy.[14] Iron exhibits two very stable redox states with a high redox potential of $E°$ (Fe^{2+}/Fe^{3+}) = +0.77 V *vs.* NHE compared to other bioavailable transition metals. Besides, the Fe^{1+} and Fe^{4+} (ferryl) states are also accessible which, for example, have been observed as intermediates in the catalytic cycles of the [FeFe] hydrogenase (Fe^{1+}) and several oxygenases (Fe^{4+}).[15,16] The Fe^{2+}/Fe^{3+} couple is at the upper end of the physiological redox range described by water oxidation (+0.82 V *vs.* NHE) and reduction (−0.40 V *vs.* NHE) at pH 7. However, the redox potential of iron can be shifted (or tuned) over a wide range by the number and nature of coordinating ligands and the resulting coordination geometry.[17] Fe^{2+} and Fe^{3+} usually prefer an octahedral geometry

for O- and N-donor amino acids and a tetrahedral geometry for S-donor amino acids. Nevertheless, both redox states can also exhibit fivefold trigonal bipyramidal or square pyramidal coordination geometries.[8,18] The flexibility is of particular value for the active sites of enzymes, as geometric preorganization of the catalytic transition-state lowers the reorganization energy. This energy is required for geometric changes between the reduced and oxidized state and is again split up into an inner contribution λ_I (changes at the metal site) and an outer contribution λ_O (changes of the surrounding protein and solvent environment).[14] For example, theoretical calculations for two tetrapyrrole cofactors, heme (Fe) and cobalamin (Co), showed for the low-spin Fe^{2+}/Fe^{3+} redox couple a much smaller inner-sphere reorganization energy than for the corresponding low-spin Co^{2+}/Co^{3+} couple.[19] Similar calculations for mono- and binuclear iron-sulfur clusters with Fe, Mn, Co, Ni and Cu revealed the lowest reorganization energies for iron as well.[14]

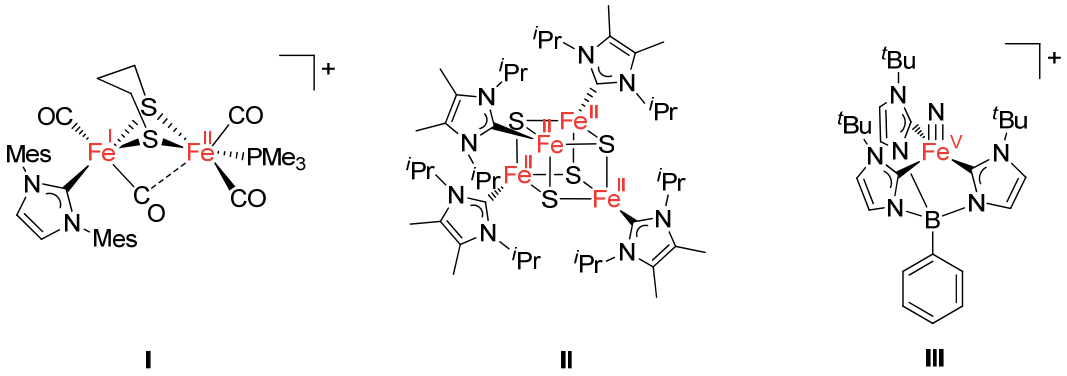
Thus, nature presumably utilized iron under a reducing atmosphere with high bioavailability. Despite challenges in acquisition, evolution did not replace the element in an oxidizing environment, due to its unique redox and coordination properties.

1.3 Bioinspired Organometallic Chemistry

Biomimetic inorganic chemistry has provided vital insights into active centers and reaction mechanisms of a large number of enzymes and cofactors.[20] Considerable effort has been devoted to the synthesis of analogues of putative intermediates involved in the catalytic cycles of enzymes.[21-24] X-ray diffraction data and spectroscopic characteristics of metalloproteins often do not allow the structural and electronic elucidation of active sites unambiguously. Synthetic low-molecular weight complexes therefore serve as more easily characterized models that provide valuable information for the interpretation of native protein data. In synthetic analogues of protein-bound active centers, the amino acids are typically mimicked by organic ligands with the same donor atoms (*e.g.* imidazole for histidine, thiolate for cysteine, carboxylate for aspartate and glutamate) in order to create model compounds with high fidelity. Besides great achievements in biomimetic inorganic chemistry, remarkable models were also reported in bioinspired organometallic chemistry;[25] organometallic compounds being defined by direct metal-carbon bonds. In this respect, carbon monoxide, cyanide and abiological *N*-heterocyclic carbene (NHC) ligands have been proven to effectively stabilize complexes with very low- or high-valent oxidation states which may otherwise not have been isolated with standard N-, O- or S-donor ligands. In general, organometallic motifs are rare in biology.[26,27] However, one of the most extensively studied metalloproteins, vitamin B_{12} (and its derivatives methylcobalamin and coenzyme B_{12}), feature organometallic Co^{III}–C units with cyanide, methyl, or 5-desoxyadenosyl ligation.[28] Furthermore, three types of hydrogenase enzymes con-

tain CO and/or CN^- ligands (see Chapter 2.1).[29] Methyl-coenzyme M reductase and carbon monoxide dehydrogenase are also known to form nickel-carbon bonds.[27] The [FeMo] cofactor of nitrogenase – the place of nitrogen reduction − is probably the most unusual bioorganometallic motif, as it contains a carbide atom (C^{4-}) coordinated by six iron atoms according to recent studies.[7]

By far the largest number of biomimetic or bioinspired organometallic complexes has been reported for hydrogenases,[15] since CO ligands are well-established in coordination chemistry. In the framework of this work though, only bioinspired NHC-coordinated complexes will be discussed. For example, an [FeFe] hydrogenase model complex with one NHC and four CO ligands (**I** in Figure 1.1) was synthesized by DARENSBOURG and coworkers.[30,31] This mixed-valent Fe^IFe^{II} complex nicely mimics the resting state of [FeFe] hydrogenases including a vacant coordination site *trans* to the bridging carbonyl. Complex **I** can also be reversibly reduced to the Fe^IFe^I state, in a similar fashion to the biological active site.[15] The high redox stability of **I** was attributed to the unique electronic and steric properties of the NHC ligand.[25,32]



I **II** **III**

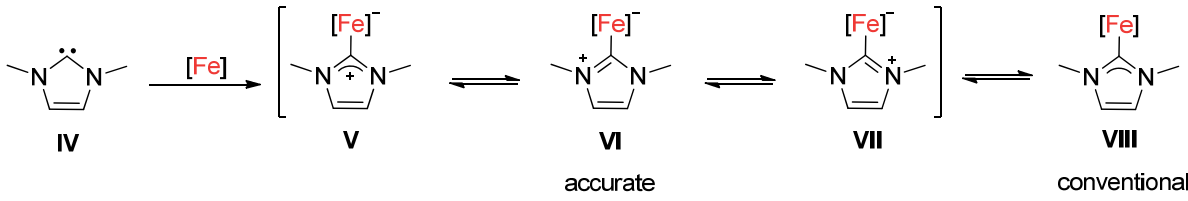
Figure 1.1: Examples for bioinspired complexes with NHC ligands.

The fourfold NHC-ligated all-ferrous [4Fe–4S] cluster **II**, which was isolated by DENG and HOLM in 2008, represents another example for bioinspired organometallic chemistry.[33] The all-ferrous redox state was also detected in the Fe-protein of nitrogenase, though coordinated by four cysteine residues. Thiolate ligands, however, are not suitable to stabilize the fully-reduced state in model systems. Nevertheless, **II** shows magnetic properties analogous to the protein-bound $[4Fe–4S]^0$ cluster.[33] This example provides proof that the magnetic interactions are not significantly influenced by the terminal ligands.[21] Complex **III** was synthesized by SMITH and coworkers in 2011 and represents the first crystallographic evidence for an iron(V) state.[34] The high-valent nitridoiron(V) unit was stabilized by a tridentate NHC ligand which once more demonstrates the remarkable properties of NHCs. High-valent oxoiron(V) intermediates have been proposed as the key intermediate in some dioxygenases,[35] thus complex **III** indicates the general existence of such species.

1.4 Iron-NHC Complexes and Their Application in Catalysis

N-heterocyclic carbenes (NHCs) have developed great popularity as ligands in organometallic chemistry and led to great advances in homogeneous catalysis in the last two decades.[36-39] On the basis of WANZLICK's pioneering investigations, ARDUENGO isolated the first free NHC in 1991.[40,41] Since then, the research on NHC complexes and their catalytic applications has extensively increased.[38] In general, NHC transition metal complexes have proven to be effective catalysts for C−C coupling reactions.[38,39,42] However, iron-NHC complexes and iron-based organometallic catalyses are still underrepresented in comparison to Ru, Pd, Rh and Ni, even though iron is more sustainable and less expensive than precious metals.[25,43] The catalytic applications of NHC complexes comprise a broad spectrum of organic transformations with the main focus on olefin metathesis and cross-coupling reactions.[37-39,42]

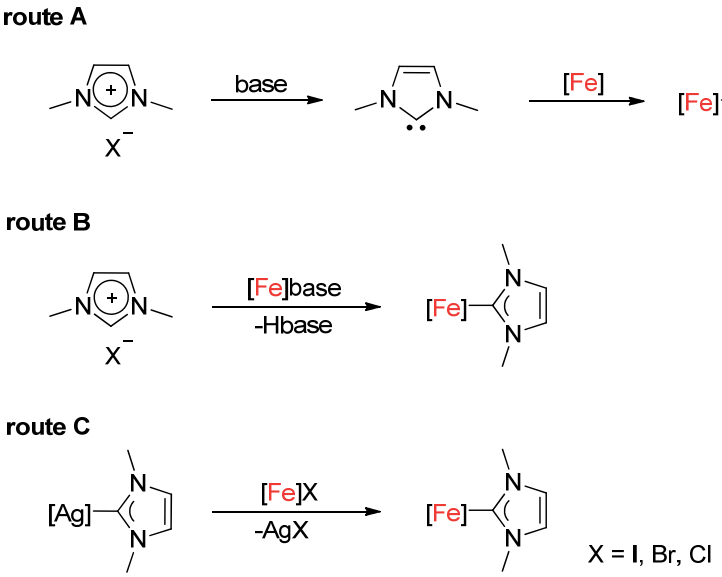
NHCs can be classified as two-electron donor ligands that exhibit strong σ-donating qualities and poor π-interactions in general.[38,39] Regarding the electronic properties, NHC ligands resemble the electron-rich phosphanes (PR_3) and cyclopentadienyls ($C_5R_5^-$) most, but cannot be considered as analogues to them.[44] The electron lone pair in NHCs is located in an energetically high σ-orbital, higher than phosphanes as, for example, PCy_3.[38,44] The energetically high π^*-orbital, on the other hand, allows $d \rightarrow \pi^*$ back-donation from filled *d*-orbitals of the metal.[39,45] In the case of electron deficient metals (*e.g.* 14 electron Rh(III) and Ir(III) complexes), NHC ligands can moreover act as $\pi \rightarrow d$ donors.[46,47] For d^6 systems like Fe(II), theoretical investigations revealed a π-contribution to the total Fe–C orbital interactions of approximately 10 %, from which approximately 80 % are caused by $d \rightarrow \pi^*$ back-donation.[47,48] Nonetheless, the σ-donor character of NHCs has a stronger influence on the complex' properties: A NHC ligand polarizes the binding metal orbital and thereby weakens the geometrically opposing bond which enhances ligand substitution kinetically.[44] This *trans*-effect is of particular importance in catalytic applications to enable substrate coordination. In catalysis, NHCs commonly adopt the role of spectator ligands as they rarely undergo modification during a reaction sequence.[44]



Scheme 1.1: Resonance structures of an iron-NHC complexes, adapted from CAZIN.[38]

The most frequently encountered type of NHC ligand is based on the imidazole scaffold. In NHC complexes, imidazolylidene ligands (*e.g.* **IV** in Scheme 1.1) can be described by several resonance structures (**V**–**VIII**). In addition to those shown in Scheme 1.1, many more variants

have been used in the literature.[38] The most accurate representation is depicted in **V**, **VI** or **VII,** with a positive charge at the ligand and a negative charge at the metal site.[38] However, the delocalized structure **VIII** is most common in the literature and will therefore also be used in this work.



Scheme 1.2: Typical routes for the synthesis of iron-NHC complexes.

Imidazolium salts, the precursors of imidazolylidene ligands, can be easily prepared from basic imidazoles or in multi-component reactions from glyoxal (or a glyoxal derivative), an amine and formaldehyde.[38,49] For the generation of NHC complexes various synthetic strategies are available.[38,50,51] The synthesis of iron-NHC complexes is usually performed *in situ* (Scheme 1.2). Either the imidazolium salt is deprotonated with an external base and subsequently treated with a metal complex (route A), or the imidazolium salt is directly reacted with an iron precursor that contains a basic ligand (route B).[25] Few iron-NHC complexes are synthesized by the transmetalation of silver-NHC precursors (route C).[52,53] While the first iron-NHC complexes were mainly prepared *via* route A,[54,55] most recent examples have mainly been reported pursuing route B.[56-61]

Despite the fact that the first iron-NHC complexes were reported in the 1970s,[62-64] the chemistry has primarily emerged in the last two decades, with major contributions in the last years. The array of iron-NHC complexes includes examples with mono-, bi-, tri- and tetradentate NHC ligands, as well as one example with a macrocyclic NHC ligand (Figure 1.2).[25] The majority of these NHC complexes contain iron(II) ions except for rare examples with iron(0),[65,66] iron(I),[32] iron(III),[67] iron(IV)[68,69] and iron(V).[34] Iron complexes with monodentate NHC ligands of type **IX** have been reported as three-, four-, five- and six-coordinated motifs, often in combination with other strong donor ligands like CO, NO^- or Cp^-. For more details in this matter the review by INGLESON and LAYFIELD is strongly rec-

ommended.[25] Selected examples of iron complexes with chelating NHC ligands used bidentate methylene-bridged bis(imidazolylidene) (**X**),[52,57,70] tridentate 2,6-bis-(imidazolylidene)pyridine (**XI**),[55,71] tris(imidazolylidene)borate (**XII**),[67,69,72,73] and tris(imidazolylidene)triethylamine (**XIII**)[68] ligands, as well as the tetradentate macrocyclic scaffold **XIV**.[74]

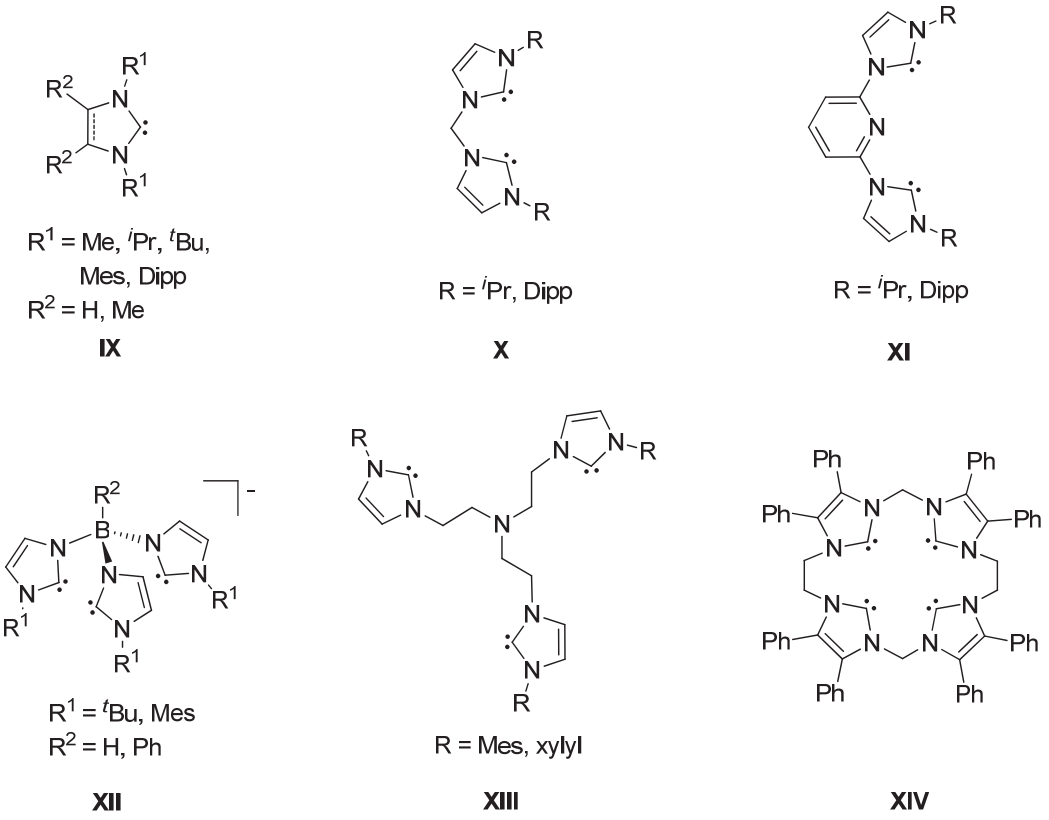


Figure 1.2: NHC scaffolds reported for iron-NHC complexes.

In organometallic catalysis, complexes with monodentate NHC ligands (**IX**) are predominantly employed.[38] Chelating NHC ligands have become more popular recently due to the higher stability of the corresponding catalysts.[75,76] Bidentate methylene- and ethylene-bridged bis(imidazolylidene) ligands (**X**), for example, are frequently used in palladium cross-coupling catalysis (*e.g.* Heck or Suzuki reactions).[77-79] Atom transfer radical polymerization was the first catalytic application with an iron-NHC complex, reported by GRUBBS in 2000 (for structure see Figure 3.4).[54] Further organic transformations catalyzed by iron-NHC complexes comprise the Kumada-type aryl–aryl cross-coupling,[80] the borylation of arenes,[56,81] the hydrosilylation of aldehydes and ketones,[82,83] the aziridination of alkenes,[74] and the cross-coupling of aryl Grignards–alkyl halides.[84,85] In many cases, iron-NHC catalysts outperformed their iron-phosphane analogues in activity or showed fundamentally different reactivities compared to the catalysts with phosphane ligands.[25] The aryl Grignard–primary (or secondary) alkyl halide cross-coupling with an iron-NHC led to substantially smaller amounts of the β-hydride eliminated byproduct in comparison with palladium cata-

lysts.[84] Similarly, lower amounts of the Grignard homo-coupled byproduct were formed in the Kumada-Corriu aryl–aryl coupling if iron-NHC catalysts were used instead of iron-phosphane catalysts.[80] The aziridination of alkenes by azides led to high activities at low catalyst loadings with a macrocyclic tetracarbene iron(II) complex (scaffold **XIV**).[74] The catalyst could even be recycled afterwards. Notably, an iron(III)- or iron(IV)imido intermediate was detected in ESI-MS which gave tentative information about the mechanism of this reaction.[74]

In summary, iron-NHC catalysts revealed superior activities or different reactivities in various reactions compared to their iron-phosphane analogues. For the coupling of aryl Grignards to alkyl halides, BEDFORD *et al.* attributed the improved catalyst properties to the stronger electron-donating ability of NHCs (*vs*. phosphanes) which leads to a more reducing Fe(II) center.[25,84] However, further mechanistic studies on iron-NHC catalysis are required for a more precise interpretation. The active species of most iron-NHC-catalyzed reactions are yet unknown.[25] In any case, the potential of iron-NHC catalysis has been demonstrated and future research holds the potential to create new catalysts and applications.

2 Bioinspired [NiFe] Hydrogenase Models

2.1 Introduction

Hydrogenases are metalloenzymes catalyzing the oxidation of molecular hydrogen (H_2) to protons and electrons and the reverse reaction. These proteins play an important role in the energy metabolism of many microorganisms, in particular archaea and bacteria, but also in some eukaryotes.[29,86] Metabolisms involving hydrogenase activity are manifold, some prominent examples are methanogenic, acetogenic, nitrogen-fixing, photosynthetic and sulfate-reducing bacteria.[87,88] Presumably, the genesis of hydrogenases dates back to primeval times when H_2 was ubiquitous in a reducing atmosphere.[89] Ancient organisms may have used H_2 as energy source, alternatively, H_2-generating prokaryotes could also have set up the hydrogen ecosystem since most hydrogenases from today's microorganisms are able to work in either direction.[29] Even though the reversible formation of H_2 from two protons and two electrons is one of the simplest reactions, the conversion requires highly specialized enzymes with complex active centers. The $E°(H_2/H^+) = -414$ mV standard redox potential makes the conversion energetically difficult for typical redox mediators as nicotinamide adenine dinucleotide (NAD) or flavin adenine dinucleotide (FAD), since the standard redox potentials of $NADH/NAD^+$ and $FADH_2/FADH^+$ are only −320 and −220 mV, respectively.[90] Oxidation of NADH or even $FADH_2$ coupled to proton reduction can only take place if the conversion is exergonic.[90] Such conditions exist in hydrogenases at low partial hydrogen pressure – usually below 10 Pa – so that $E(H_2/H^+)$ rises near to −300 mV, thus being in the physiologically feasible range.[91,92]

Hydrogenases were first identified by STEPHENSON and STICKLAND in 1931.[93] However, chemistry in this field started evolving after the first structural elucidations of [NiFe] and [FeFe] enzymes by X-ray crystallography about only two decades ago.[94,95] After this, the number of publications increased rapidly. Meanwhile, excellent reviews give insight into the physiological,[29,86,90,92] structural,[96-98] spectroscopic[99-101] and electrochemical properties[102] of hydrogenases. The huge interest in hydrogenases rests on the idea of H_2 as environmentally clean energy carrier, as it is considered to be the ideal CO_2-free alternative to fossil fuels if produced in a sustainable manner (*e.g.* by sunlight-driven photolysis of water).[103] Present techniques for the industrial synthesis of H_2 comprise steam reforming and partial oxidation processes of fossil resources. These processes are energetically inefficient and release CO_2. The utilization of H_2 in fuel cells on the other side involves rare precious metal catalysts like platinum and palladium.[103] Consequently, new catalysts for large-scale H_2 production and cleavage are required, ideally on the basis of inexpensive and abundant metals like iron and/or nickel.[103] Most hydrogenases work at ambient temperature and pressure with high turnover rates and therefore provide features desired for future catalysts.[103] Elucidation of the struc-

tural and mechanistic characteristics of hydrogenases is the first step, the second would be to incorporate these properties into low-molecular weight analogues.[15,92,104] Equally suitable systems would be *in vivo* or *in vitro* catalysts, but the utilization of hydrogenases was so far hindered by their high dioxygen-sensitivity. However, some remarkably dioxygen-tolerant [NiFe] hydrogenase variants have been identified recently that work in the presence of O_2 and even CO and H_2S (Pt-catalyst inhibitors).[98,102] In summary, hydrogenases are able to activate molecular hydrogen under mild conditions or generate H_2 efficiently and thus are of great interest from both commercial and scientific viewpoints.[105]

2.1.1 Classification of Hydrogenases

Three phylogenetically distinct classes of hydrogenases are known so far. With respect to their active centers they are named nickel-iron [NiFe], iron-iron [FeFe] and iron-sulfur cluster-free [Fe] hydrogenase (Figure 2.1).[29,92] Although the three enzyme classes do not share any sequence similarity, a common structural feature is the presence of iron with at least one carbonyl ligand at the active site.[92,106] Hydrogenases display rare examples of organometallic motifs in nature despite the high toxicity of CO and CN^- in biological systems.

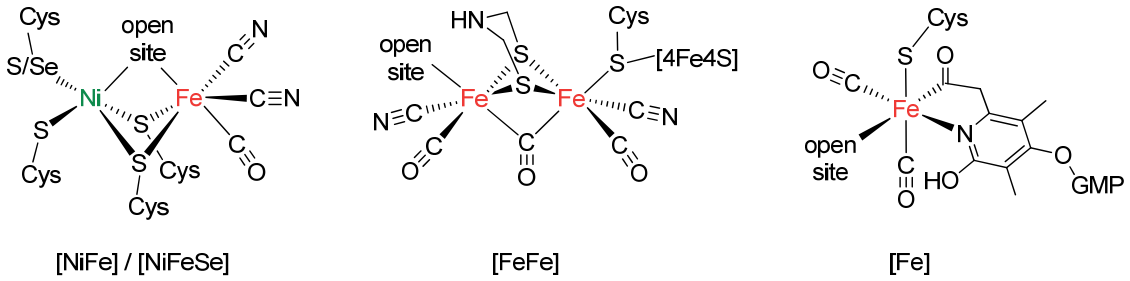


Figure 2.1: Active centers of the three hydrogenase classes which catalyze the interconversion of molecular hydrogen and protons and electrons. The [Fe] hydrogenase active site (right) is depicted without its 5,10-methenyltetrahydromethanopterin cofactor; the pyridine ligand is linked to a guanosine monophosphate (GMP) moiety.[92,98]

The active centers of [NiFe] and [FeFe] hydrogenases consist of twice thiolate-bridged binuclear metal cores,[96] while [Fe] hydrogenases contain a mononuclear iron site and an organic cofactor.[107,108] Furthermore, [NiFeSe] homologues to [NiFe] hydrogenases are known in which one terminal cysteine is replaced by selenocysteine that show even higher catalytic activity.[109,110] There has been an intense discussion about the identity of the central heteroatom in the bridging dithiolate ligand of [FeFe] hydrogenases. According to recent experimental data and theoretical investigations, nitrogen is most likely (Figure 2.1).[111,112] Both [NiFe] and [FeFe] hydrogenases promote the interconversion of H_2 and protons and electrons in either direction *via* a heterolytic mechanism ($H_2 \rightleftharpoons H^+ + H^- \rightleftharpoons 2H^+ + 2e^-$). However, one direction is usually preferred and imposed by the enzyme environment: [NiFe] hydrogenases tend to H_2 oxidation while [FeFe] hydrogenases more often catalyze H_2 production.[106] In