

# Insight into the Iron–Molybdenum Cofactor of Nitrogenase from Synthetic Iron Complexes with Sulfur, Carbon, and Hydride Ligands

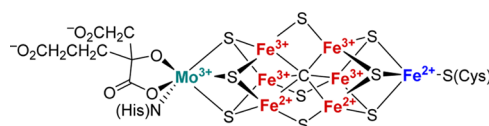
Ilija Čorić and Patrick L. Holland\*

Department of Chemistry, Yale University, New Haven, Connecticut 06520, United States

**ABSTRACT:** Nitrogenase enzymes are used by microorganisms for converting atmospheric  $N_2$  to ammonia, which provides an essential source of N atoms for higher organisms. The active site of the molybdenum-dependent nitrogenase is the unique carbide-containing iron–sulfur cluster called the iron–molybdenum cofactor (FeMoco). On the FeMoco,  $N_2$  binding is suggested to occur at one or more iron atoms, but the structures of the catalytic intermediates are not clear. In order to establish the feasibility of different potential mechanistic steps during biological  $N_2$  reduction, chemists have prepared iron complexes that mimic various structural aspects of the iron sites in the FeMoco. This reductionist approach gives mechanistic insight, and also uncovers fundamental principles that could be used more broadly for small-molecule activation. Here, we discuss recent results and highlight directions for future research. In one direction, synthetic iron complexes have now been shown to bind  $N_2$ , break the N–N triple bond, and produce ammonia catalytically. Carbon- and sulfur-based donors have been incorporated into the ligand spheres of Fe– $N_2$  complexes to show how these atoms may influence the structure and reactivity of the FeMoco. Hydrides have been incorporated into synthetic systems, which can bind  $N_2$ , reduce some nitrogenase substrates, and/or reductively eliminate  $H_2$  to generate reduced iron centers. Though some carbide-containing iron clusters are known, none yet have sulfide bridges or high-spin iron atoms like the FeMoco.

## 1. INTRODUCTION

Nitrogen is an essential element for all life, and the supply of biologically available nitrogen limits the productivity of many terrestrial and marine ecosystems.<sup>1–3</sup> The most plentiful source of nitrogen is  $N_2$ , but this molecule has low reactivity. Biological fixation of  $N_2$  to ammonia,<sup>4–8</sup> a bioavailable source of nitrogen, is performed by diazotrophic microorganisms.<sup>9–11</sup> These specialized microorganisms can be free-living or in symbiotic relationships with certain plants (e.g., legumes) and animals (e.g., termites).<sup>12,13</sup> Before the invention of the Haber–Bosch process for industrial ammonia production, most nitrogen atoms in living organisms originated from these microorganisms.<sup>14,15</sup> They have the only type of enzymes that can perform the key  $N_2$ -reducing reaction, and these are termed nitrogenase enzymes. Elucidation of the mechanism of biological  $N_2$  fixation by nitrogenases is a great challenge in bioinorganic chemistry. Chemists aim to explain the mechanism of  $N_2$  reduction in nature and are also excited about the opportunity to harness the power of nitrogenase for other applications.



**Figure 1.** Structure of the resting state FeMoco from high-resolution X-ray crystallography.<sup>16</sup> The oxidation states of the iron atoms shown here are from X-ray absorption and anomalous dispersion studies.<sup>17,18</sup> The belt iron sites are colored red.

$N_2$  reduction by nitrogenases occurs at metal clusters, with the most well-characterized being the iron–molybdenum cofactor (FeMoco) (Figure 1).<sup>6,16,19–21</sup> FeMoco contains seven Fe and one Mo that are connected by bridging sulfides and arranged around a central carbide. The biosynthesis of FeMoco from two  $Fe_4S_4$  clusters, an additional S atom, and a C atom initially forms an  $Fe_8S_9C$  carbide cluster as an intermediate.<sup>22,23</sup> Substitution of an apical Fe for Mo/homocitrate is followed by the transfer of the cluster to the apo form of the catalytic MoFe protein.

Alternative nitrogenases use V or Fe in place of Mo, and are less efficient at  $N_2$  fixation than the Mo-dependent enzyme.<sup>24–26</sup> X-ray structures of the alternative nitrogenases are not yet available, but biochemical and genetic studies indicate that the structures of their active site cofactors are similar to the FeMoco.<sup>24,25,27</sup> Spectroscopic studies further support the idea that the iron–vanadium cofactor (FeVco) contains an interstitial carbide like the FeMoco.<sup>28,29</sup> The shared cluster type in various nitrogenases, and studies on the activity and spectroscopy of variants of the FeMo enzyme that derive from point mutations,<sup>30–33</sup> indicate that the belt iron sites (indicated in red in Figure 1) are the site of  $N_2$  binding and reduction.<sup>6,32,34</sup>

Well-defined synthetic “model” systems are often used to provide insight into the feasibility of different mechanisms and structures within enzymes.<sup>35,36</sup> However, the chemistry of iron complexes in a coordination environment like the Fe sites in the FeMoco was unknown until a number of recent advances. Here we discuss the implications of recent progress in synthetic iron complexes supported by ligand scaffolds that coordinate to iron through carbon and/or sulfur, as well as iron hydrides that are relevant to the mechanism of  $N_2$  reduction.<sup>6</sup> Reviews discussing  $N_2$  reduction by Mo systems, by Fe systems with P and N ligands, and by other metals are available elsewhere.<sup>37–44</sup>

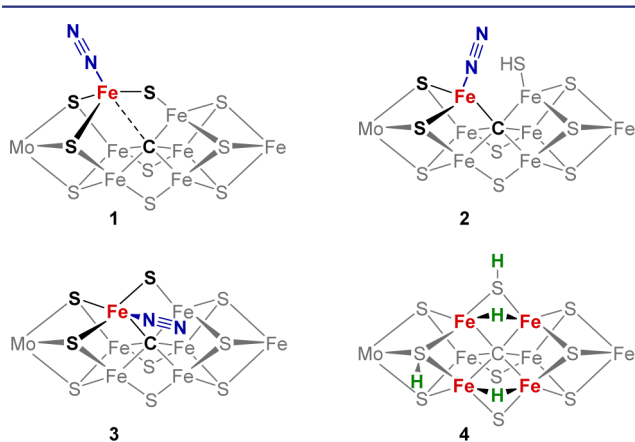
Received: January 21, 2016

Published: May 12, 2016

## 2. COORDINATION CHEMISTRY OF FeMoco

Recent attention in the nitrogenase modeling community has focused on the belt Fe sites (see Figure 1), each of which has pseudo-tetrahedral 3S-1C coordination in the resting state. X-ray crystal structures are known for nitrogenases from both *Azotobacter vinelandii* and *Clostridium pasteurianum* to resolutions of better than 1.1 Å, and the atomic positions in the FeMoco in each were determined to high precision (~0.02 Å).<sup>16,45</sup> The Fe–S bond lengths are all the same within ±0.03 Å, suggesting that none of them are protonated. The FeMoco is mixed-valent in the resting state with both Fe<sup>2+</sup> and Fe<sup>3+</sup> centers, which couple to give an *S* = 3/2 ground state.<sup>6,7</sup> A combination of X-ray absorption spectroscopy and computations show that the oxidation states are Fe<sup>2+</sup><sub>3</sub>Fe<sup>3+</sup><sub>4</sub>Mo<sup>3+</sup> in the resting state.<sup>17,46,47</sup> Spatially resolved anomalous dispersion refinement of the structure recently confirmed this picture.<sup>18</sup> These data suggest the oxidation state assignments indicated in Figure 1, and the three sites with higher electron density are on the side of the cofactor that abuts two positively charged arginine residues. Thus, even though the FeMoco has near three-fold symmetry in the core bonding, there is asymmetry imposed by the local polarity in the protein.

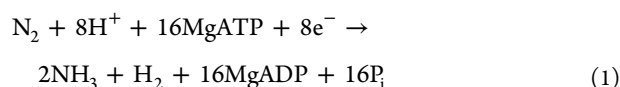
Though this detailed characterization of the resting-state geometric and electronic structure is a major achievement, the binding of substrates occurs only after conversion of the FeMoco to other, more reduced forms that have not been structurally characterized.<sup>6</sup> The cofactor is connected to the protein through only two linkages, and there are many precedents for structural rearrangements in synthetic iron–sulfur clusters,<sup>48,49</sup> and therefore the Fe–C–S cluster structure is likely to rearrange during the catalytic cycle. Binding of N<sub>2</sub> to Fe sites is implicated most strongly (see above), so most chemists have considered that breaking or elongation of the bonds to belt Fe atoms could accompany or precede substrate binding. Consequently, N<sub>2</sub> binding proposals include either elongation/dissociation of an Fe–C bond (e.g., 1, Figure 2),<sup>50–53</sup> or Fe–S bond cleavage with protonation of a sulfide (e.g., 2, Figure 2).<sup>54–58</sup> Supporting 1, extended X-ray absorption fine structure (EXAFS) and nuclear resonance vibrational spectroscopy (NRVS) studies indicate the lengthening of Fe–C bond in FeMoco upon binding of propargyl alcohol.<sup>59</sup> In Fe-phosphine model complexes (described below), Fe–C bond elongation has been observed upon



**Figure 2.** Possible FeMoco intermediates during reduction of N<sub>2</sub>. Additional potential protonation sites and cluster charges are not shown.

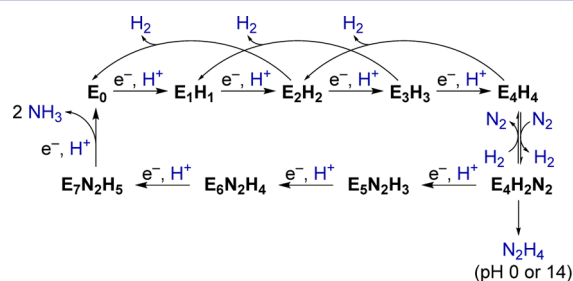
reduction and N<sub>2</sub> binding.<sup>60,61</sup> Supporting 2, Fe–S bond cleavage in FeMoco is experimentally supported by the observation of sulfide replacement by CO and Se in the structures of nitrogenase treated with CO or selenocyanate,<sup>62</sup> by Fe–S cleavage in smaller Fe–S clusters,<sup>63,64</sup> and by observation of Fe–S cleavage with N<sub>2</sub> binding in a Fe model complex.<sup>65</sup> Another plausible geometry for N<sub>2</sub> binding is *endo* coordination, where N<sub>2</sub> is positioned close to three additional iron atoms (3, Figure 2).<sup>6,66</sup> Other hypotheses for N<sub>2</sub> binding modes include η<sup>2</sup> coordination and bridging modes.<sup>57,66</sup> End-on binding of N<sub>2</sub> has been proposed on the basis of ENDOR studies of species trapped during N<sub>2</sub> reduction by nitrogenase, which show coupling to only one type of N environment.<sup>67</sup>

N<sub>2</sub> reduction by Mo-dependent nitrogenase always releases at least 1 equiv of H<sub>2</sub> per N<sub>2</sub> reduced.<sup>68,69</sup> The dependence of the N<sub>2</sub> reduction rate on [N<sub>2</sub>] suggests the limiting stoichiometry in eq 1 (P<sub>i</sub> = inorganic phosphate-containing



products).<sup>7,68,70</sup> Note that this stoichiometric reaction is highly exergonic because of the consumption of so much ATP.<sup>71,72</sup> The overall energy efficiency of N<sub>2</sub> reduction is low, which reflects the challenging kinetic problems for performing this multi-step reaction under ambient conditions.

Mechanistic data on Mo-nitrogenase are commonly rationalized using the Thorneley–Lowe kinetic scheme, which was derived from global fitting of data from kinetic studies on the enzymatic reaction.<sup>70,73–76</sup> In this scheme, intermediates are named E<sub>*n*</sub>, where *n* represents the number of electrons added to the resting state. Importantly, the kinetic data indicate that N<sub>2</sub> binding does not take place until three or four electrons have been added. A series of EPR/cryoannealing studies confirm this model, and specifically show that E<sub>4</sub> can reversibly bind N<sub>2</sub> to form a spectroscopically observed intermediate.<sup>77,78</sup> Figure 3 shows a version of the Thorneley–Lowe scheme with binding of N<sub>2</sub> at the E<sub>4</sub> state.<sup>6</sup>



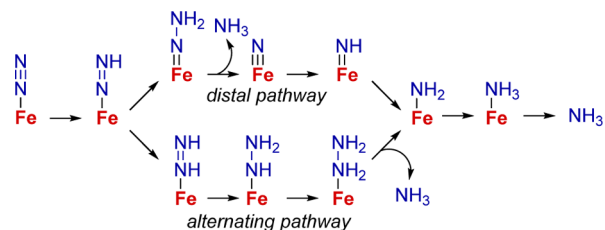
**Figure 3.** Simplified Thorneley–Lowe scheme for N<sub>2</sub> reduction.

One frustrating aspect of nitrogenase research is that none of the redox potentials for E<sub>*n*</sub> transformations are known.<sup>79</sup> The electrons are supplied by the “Fe protein” which has an iron–sulfur cluster whose potential is influenced by the binding of ATP, ADP, and the catalytic protein.<sup>80</sup> The addition of the Fe protein in the absence of substrates inevitably leads to production of H<sub>2</sub>, rendering the electrochemistry irreversible.<sup>7</sup> However, the use of the same reductant for all eight electrons is an important constraint, implying that there is not excessive charge buildup on the cofactor.<sup>6,81,82</sup> This feature has traditionally been explained by assuming that one proton is

added per electron, as shown in Figure 3. This is an example of proton-coupled electron transfer (PCET), where the negative charge of each electron is balanced by the positive charge of a proton.<sup>83,84</sup>

ENDOR studies suggest that the  $E_4$  state (presumably protonated to give  $E_4H_4$ ) has two bridging hydrides, implying that there are two additional protons that reside on the sulfide bridges.<sup>85–91</sup> One potential structure for  $E_4H_4$  that satisfies the experimental constraints is shown as 4 in Figure 2.<sup>88</sup> Interestingly, the  $4H^+/4e^-$  can be lost from  $E_4$  as two molecules of  $H_2$  to regenerate the resting state, even in frozen solution.<sup>87,90</sup> The fact that  $H_2$  is a competitive inhibitor of  $N_2$  reduction by the enzyme, that  $D_2$  can be incorporated into certain substrates,<sup>7,78</sup> and that higher  $N_2$  pressures lead to higher concentrations of  $E_4H_2N_2$ ,<sup>77</sup> support the idea that reversible  $H_2$  loss is an integral part of binding of  $N_2$  by nitrogenase.<sup>6,70,77,78</sup> Although this reductive elimination of  $H_2$  “wastes” two reducing equivalents, it might be the only way under biological constraints to create a reduced iron site that can bind  $N_2$  strongly.<sup>55,77</sup> Fryzuk and Quadrelli have reviewed the ability of hydrides to store electrons for  $N_2$  activation without formal reduction of the metal.<sup>81,82</sup> Interestingly, the reductive elimination of  $H_2$  does not occur until  $N_2$  binding, as shown by the fact that  $H_2/D_2$  exchange only occurs in the presence of  $N_2$ .<sup>7,78</sup>

After  $N_2$  binding, multistep delivery of electrons and protons to  $N_2$  could give a range of potential intermediates. Two mechanistic pathways, distal and alternating,<sup>34</sup> are commonly invoked as possibilities (Figure 4), and are distinguished by the location of protons on the  $N_2$  unit and by the timing of ammonia release.<sup>6</sup> An alternating pathway for  $N_2$  reduction on iron is supported by computational, spectroscopic, and trapping studies on the enzyme.<sup>34,92–97</sup> The alternating mechanism implicates hydrazine ( $H_2N-NH_2$ ) and diazene ( $HN=NH$ ) complexes as intermediates, consistent with the observations that these two molecules are substrates for nitrogenase.<sup>93,95–97</sup> Though Figure 4 shows intermediates with only one iron center, many of the  $N_xH_y$  species along the alternating pathway could be bridging (see below), so more than one iron center could well be involved in the reduction. In addition, hybrid mechanisms could involve intermediates from both alternating and distal pathways, by addition of protons or transfer of H atoms in intermediates.<sup>61,98,99</sup>



**Figure 4.** Potential intermediates in  $N_2$  reduction on iron. All steps except second  $NH_3$  release include input of  $H^+$  and  $e^-$ .

Nitrogenases are capable of reducing a variety of other unsaturated compounds including CO and  $CO_2$ .<sup>100</sup> Vanadium-dependent nitrogenase is especially efficient for reductive coupling of CO to make C–C bonds,<sup>101,102</sup> a process related to Fischer–Tropsch synthesis.<sup>103</sup> Even isolated cofactors display the ability to form C–C bonds from CO.<sup>104</sup> The coordination chemistry of FeMoco with CO and  $CO_2$  is relevant to

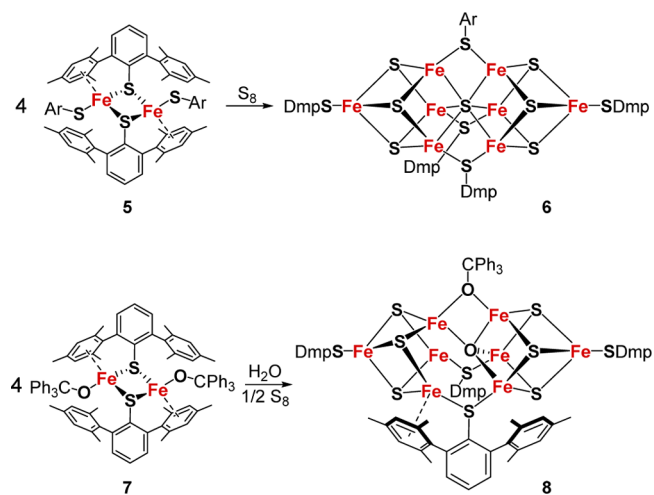
understanding the mechanism behind these potentially useful processes and aids the study of CO inhibition of the enzyme, and has been discussed in detail elsewhere.<sup>62a,105</sup>

### 3. SYNTHETIC IRON–SULFUR CLUSTERS

Our description of synthetic compounds will start with the most obvious feature of FeMoco: that it is an iron–sulfur (Fe–S) cluster. Fe–S clusters are found in a range of different kinds of enzymes and electron-transfer proteins.<sup>106,107</sup> In order to understand their properties, Fe–S clusters have been synthesized, often through assembly from simple starting materials.<sup>48,49,108</sup> Artificial clusters of many sizes and topologies are now accessible, and heterometals can be incorporated into clusters. However, no Fe–S cluster with a carbide has yet been synthesized by chemists.

Ohki, Tatsumi, and co-workers reported several examples of iron–sulfur clusters that have a topology reminiscent of FeMoco but with a central sulfide or oxide (Figure 5).<sup>109–111</sup>  $Fe_8S_7$  cluster 6 was formed from the reaction of coordinatively unsaturated dinuclear  $Fe^{2+}$  complex 5 with  $S_8$  in toluene.<sup>109</sup> In this remarkable self-assembly process, some  $Fe^{2+}$  centers in the starting material are oxidized to give a  $Fe^{2+}_3Fe^{3+}_3$  cluster. The Fe centers in 6 are arranged in two  $Fe_4S_3$  units that are connected by three thiolate bridges and a central  $\mu^6$ -sulfide. A  $Mo_2Fe_6S_9$  cluster which has two cuboidal  $MoFe_3(\mu^3-S)_3$  units joined via a central  $\mu^6$ -S atom was reported by Holm.<sup>112</sup> Selectively joining  $MoFe_3S_3$  and  $Fe_4S_3$  subclusters in structures similar to FeMoco remains a challenge. It is likely that comparison of symmetrical and unsymmetrical clusters would provide insight into the role of Mo in the FeMoco.

A  $Fe_8$ -sulfur cluster (8) that contains an oxygen atom as the central atom has been synthesized by the reaction of the coordinatively unsaturated dinuclear iron(II) thiolate/alkoxide complex 7 with water and sulfur in toluene (Figure 5).<sup>111</sup> The oxygen atom in this cluster of five  $Fe^{2+}$  and three  $Fe^{3+}$  forms a bridge between two  $Fe_4S_3$  fragments. One of the exciting aspects of this cluster is the presence of two coordinatively unsaturated Fe atoms that are weakly bound to mesityl rings. Formation of such coordinatively unsaturated iron sites after the reduction of the resting state FeMoco might be responsible for initial  $N_2$  binding to the cofactor (1, Figure 2). Though cluster 8 does not react with 1 atm of  $N_2$ , its reduced forms



**Figure 5.** Structures of synthetic iron–sulfur clusters. Dmp = 2,6-(mesityl) $_2C_6H_3$ , Ar = 2,4,6- $Pr_3C_6H_2$ .



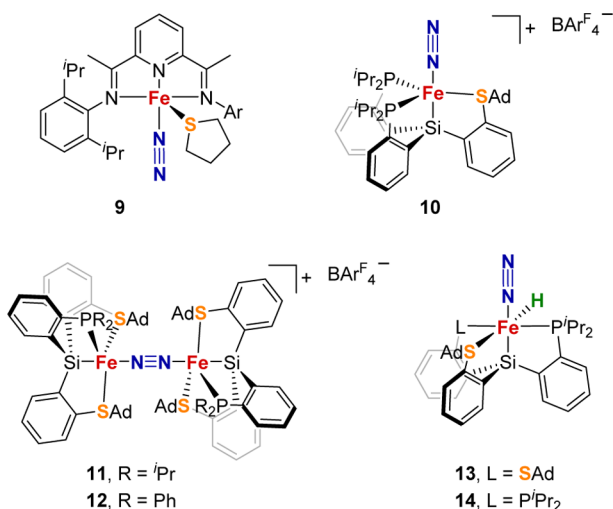
have not yet been reported and might have heightened  $N_2$  reactivity.

Clusters related to **6** but having one of the bridging thiolates replaced with an alkoxide or an amide are also known.<sup>109,111</sup> Although they do not contain Mo, the synthesis of clusters in Figure 5 demonstrates the stability of cluster structures similar to the FeMoco. They are further relevant to the proposed structure of the cofactor in all-iron nitrogenase, and to the  $Fe_8S_9C$  cluster that is formed during biosynthesis of FeMoco.<sup>22,23</sup>

Despite remarkable achievements in the synthesis of complex Fe–S clusters,  $N_2$  binding to an iron-sulfide cluster has never been reported.<sup>48,49,113–115</sup> Recently, a synthetic iron-sulfide cluster was combined with cofactor-deficient nitrogenase, which formed an artificial enzyme that reduced acetylene, but not  $N_2$ .<sup>116</sup> Pairing of synthetic and biological components has substantial promise as a strategy for identifying the essential components of the nitrogenase mechanism.

#### 4. IRON– $N_2$ COMPLEXES WITH SULFUR LIGANDS

It is well-known that the  $\sigma$  interaction of  $N_2$  with metals is weak, and that  $\pi$ -backbonding from filled metal  $d$  orbitals into the  $\pi^*$  orbitals of  $N_2$  is most influential.<sup>38</sup> Iron– $N_2$  complexes have been isolated in a number of systems where the iron atom has the +2 oxidation state or lower.<sup>39,40</sup> However, only a few synthetic complexes are known that have both sulfur and  $N_2$  ligands on the same iron center (Figure 6).<sup>117</sup> Since these resemble feasible substructures of crucial  $N_2$ -bound species in nitrogenase catalytic cycle, they have been important topics of inquiry.



**Figure 6.** Fe– $N_2$  complexes with thioether ligands.  $BAR^F_4^-$  = tetrakis(3,5-trifluoromethylphenyl)borate.

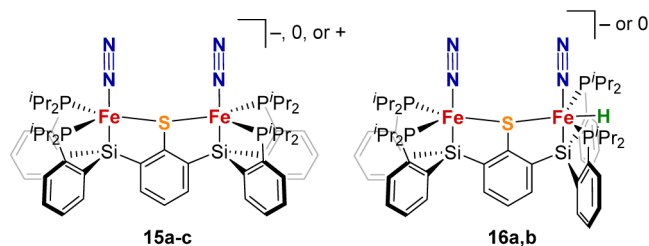
The Peters group reported a series of thioether ligated Fe– $N_2$  complexes with tetradentate ligands that are conceptually related to their previous tris(phosphine) complexes.<sup>118</sup> A ligand with one thioether and two phosphine donors yielded the cationic paramagnetic ( $S = 1$ ) complex **10** (Figure 6). The  $N_2$  ligand in **10** is lost under reduced pressure, and this lability is consistent with its high N–N stretching frequency of  $2156\text{ cm}^{-1}$ . Reduction of **10** to with potassium/graphite ( $KC_8$ ),  $Na(Hg)$ , or  $CoCp_2$  resulted in  $N_2$  loss, cleavage of the S–C(alkyl) bond, bridging S, and/or thioether dissociation. These undesired reactions illustrate common difficulties that arise

during attempts to stabilize  $N_2$  complexes using sulfur-based ligands.

When a ligand with two thioethers and one phosphine arm was used, the resulting iron(II) complex did not bind  $N_2$ . However, partial reduction of this complex with 0.5 equiv of  $Cr(C_6H_6)_2$  resulted in the mixed-valent species **11** and **12**, which have bridging  $N_2$  (Figure 6).<sup>118</sup> Compounds **10**–**12** have  $N_2$  bound to Fe that is ligated by sulfur donors, and their distorted trigonal-bipyramidal geometries model the potential binding mode **1** in the FeMoco (Figure 2).

Interestingly, a mononuclear Fe– $N_2$  species **13** with two thioether donors can be obtained by introduction of a hydride ligand at the iron center (Figure 6).<sup>118</sup> Since metal– $N_2$  interactions are so dependent on backbonding, it is reasonable that the more electron rich Fe in **13** is better able to bind  $N_2$ . A similar iron– $N_2$  hydride complex **14** was obtained using a ligand with a single thioether donor. Thus, it appears that strong-field hydride ligands can facilitate  $N_2$  binding to Fe, corresponding to one possible role for hydrides in the FeMoco.<sup>119</sup>

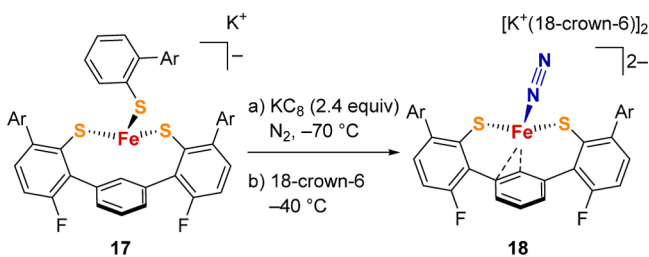
Thiolate donors, which are negatively charged, are better suited to model the anionic sulfides in the FeMoco. Peters recently described the first example of a thiolate–iron– $N_2$  species, with  $Fe^+Fe^+$ ,  $Fe^+Fe^{2+}$ , and  $Fe^{2+}Fe^{2+}$  in binuclear thiolate bridged iron complexes that are additionally coordinated by phosphine and silyl donors (**15**, Figure 7).<sup>120</sup> The anionic bis( $N_2$ ) species produces  $1.8 \pm 0.3$  equiv of ammonia when treated with excess  $KC_8$  and  $[H(OEt_2)_2]BAR^F_4$ , and the cationic  $Fe^{2+}Fe^{2+}$  species can catalyze the disproportionation of  $N_2H_4$  to  $NH_3$  and  $N_2$ . Furthermore, the same ligand scaffold enables formation of  $Fe^+Fe^{2+}$  and  $Fe^{2+}Fe^{2+}$  iron hydride species having an Fe– $N_2$  fragment (**16**, Figure 7).



**Figure 7.**  $N_2$  binding to iron using a thiolate donor.

In order to create an environment consisting only of donor atoms present in the FeMoco, the bis(thiolate) ligand platform in **17** was introduced (Figure 8).<sup>65</sup> Reduction of tris(thiolate) iron(II) complex **17** to a formal iron(0) oxidation state at low temperature resulted in the loss of one thiolate, and formation of a terminal  $N_2$  complex **18** that features partial coordination of the central arene with Fe–C distances of 2.04 and 2.24 Å (Figure 8). The iron in **18** thus has close coordination of two S and one C, as in the proposed  $N_2$  binding mode **2** for the FeMoco (Figure 2). Breaking an Fe–S bond concomitant with  $N_2$  binding during the formation of **18** suggests that breaking an Fe–S bond is a chemically reasonable route to  $N_2$  binding in the FeMoco. Furthermore, **18** has a quite activated  $N_2$  ligand, as evidenced by N–N stretching frequency of  $1880\text{ cm}^{-1}$ . This shows that thiolates give electron-rich, high-spin iron centers that backbond well into the  $\pi^*$  orbital of  $N_2$ , though  $N_2$  is lost readily from **18** at room temperature.

Future work is necessary to advance the chemistry of biomimetic complexes with S donors. First, it will be



**Figure 8.** N<sub>2</sub> binding to an iron–sulfur–carbon site. Ar = 2,4,6-*i*-Pr<sub>3</sub>C<sub>6</sub>H<sub>2</sub>.

advantageous in the future to incorporate carbides and additional iron atoms in a complex with a S-dominated ligand sphere. In addition, use of sulfides rather than thiolates or thioethers is a challenge that will require careful synthetic design. Ideally, it will be possible to design S/C ligand spheres that enable direct comparison of hypotheses 1 and 2 (Figure 2).

### 5. CARBIDE COMPLEXES, AND IRON–N<sub>2</sub> COMPLEXES WITH CARBON LIGANDS

The carbide in the FeMoco comes from the methyl group of the sulfonium ion *S*-adenosylmethionine, which is thought to be transferred to a cluster sulfur, and subsequently subjected to abstraction of H atoms.<sup>121–123</sup> The carbide is neither exchanged nor lost from the cofactor during catalysis.<sup>124</sup> The fact that there is cellular machinery devoted to the synthesis of this unusual cage structure implies that the carbide may play a functional role in N<sub>2</sub> reduction. Some possibilities for this functional role include enforcement of an appropriate geometry in the active site, stabilization of the cluster<sup>124</sup> (note that carbides are present in steel<sup>125</sup>), transient formation/cleavage of Fe–C bonds,<sup>50–53</sup> and reversible formation of C–H bonds. Recent computational studies propose that the carbide could even engage in covalent bonding with the N atoms during the N<sub>2</sub> reduction.<sup>126,127</sup>

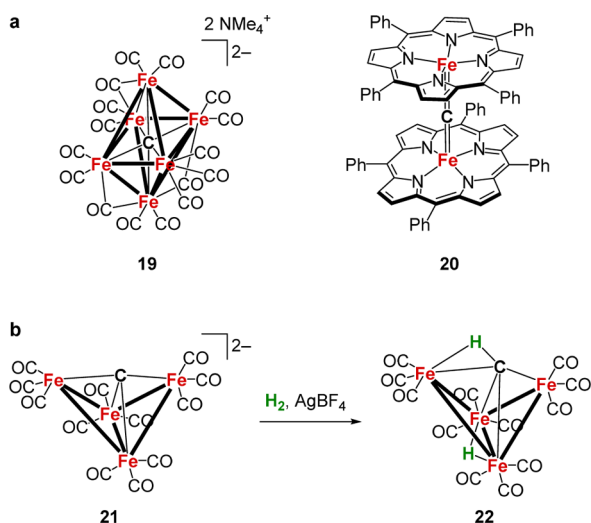
Synthetic iron-carbide clusters are also known, though in these cases the carbides come from very different sources.<sup>128–144</sup> In a six-iron example, the Fe<sub>6</sub> cluster [Me<sub>4</sub>N]<sub>2</sub><sup>+</sup>[Fe<sub>6</sub>C(CO)<sub>16</sub>]<sup>2-</sup> (**19**, Figure 9a) was studied in the early 1970s as a model of possible intermediates in the iron-catalyzed Fischer–Tropsch process.<sup>129,130</sup> In collaboration with DeBeer,

we recently reported more extensive crystallographic characterization of **19**, as well as X-ray emission studies that elucidated the electronic structure of the iron-carbide core and demonstrated the delocalized orbitals that give rise to Fe–C bonding.<sup>145</sup> Oxidation of **19** leads to related Fe<sub>5</sub> and Fe<sub>4</sub> carbide/carbonyl clusters.<sup>134,142</sup> One transformation with particular relevance to the FeMoco mechanism is that [Fe<sub>4</sub>C(CO)<sub>12</sub>]<sup>2-</sup> (**21**, Figure 9b) can be oxidized in the presence of H<sub>2</sub> to give neutral HFe<sub>4</sub>(CH)(CO)<sub>12</sub> (**22**).<sup>134,136,139,146</sup> This product can also be synthesized through double protonation of the cluster.<sup>134,140</sup> The oxidative addition of H<sub>2</sub> is the microscopic reverse of reductive elimination from a hydride–iron cluster, and suggests that reversible formation of C–H bonds in the FeMoco should be considered as a possibility.

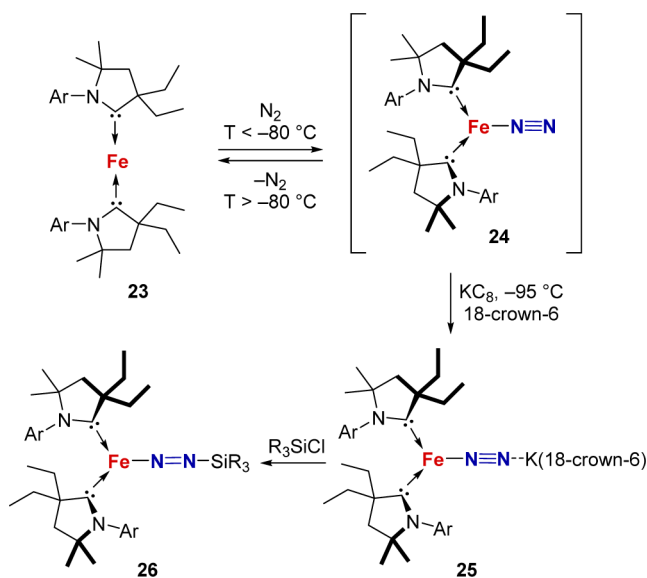
It should be borne in mind that there are several differences between these synthetic carbide clusters and the FeMoco. First, none of these carbide clusters have the trigonal prismatic geometry of the FeMoco. Second, the  $\pi$ -acidic CO ligands in the synthetic iron-carbide clusters stabilize Fe<sup>+</sup> and Fe<sup>0</sup> oxidation levels, as opposed to Fe<sup>3+</sup> and Fe<sup>2+</sup> centers that are present in the enzyme. Finally, the strong-field CO ligands in the synthetic clusters make them diamagnetic, which contrasts to the sulfide-supported, high-spin iron sites in the enzyme.<sup>17,18,47</sup> Thus, the behavior of these CO-supported clusters may not be representative of the FeMoco environment. The synthesis of iron-carbide clusters in weak-field environments (preferably with S donors) is needed in order to predict the chemical behavior of Fe atoms in the FeMoco.

In the above carbonyl clusters, the carbide is derived from reduction of a coordinated CO, and thus it is probable that new synthetic strategies will be needed for non-carbonyl clusters. Several  $\mu$ -carbido complexes with iron are known, although their chemistry has not been explored extensively.<sup>147–153</sup> The diamagnetic tetraphenylporphyrinato complex **20** with a linear Fe=C=Fe bridge was theoretically predicted and synthesized in the early 1980s (Figure 9a).<sup>147,148,154</sup> The carbide in **20** originates from Cl<sub>4</sub> in a one-step reaction, suggesting that this route might be applicable to other carbides in non-carbonyl environments.

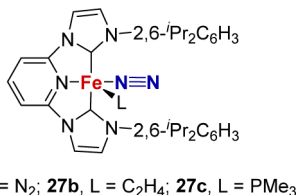
Because of the difficulty in designing appropriate clusters, simpler carbon-based donors have been used to make mononuclear complexes. One compelling choice of C-donor is the *N*-heterocyclic carbene (NHC), which can form stable complexes with iron.<sup>155</sup> However, NHC-stabilized Fe–N<sub>2</sub> complexes are rare.<sup>156</sup> Peters reported that a two-coordinate (CAAC)<sub>2</sub>Fe complex **23** [CAAC = cyclic (alkyl)(amino)-carbene] binds N<sub>2</sub> at low temperature to form a putative three-coordinate N<sub>2</sub> complex **24** (Figure 10).<sup>157</sup> It can be reduced to Fe(-I) in the heterobimetallic bridging N<sub>2</sub> complex **25**, which has a low N–N stretching frequency of 1850 cm<sup>-1</sup>. Three-coordinate Fe–N<sub>2</sub> complexes are rare and have previously been observed using bulky  $\beta$ -diketiminato ligands.<sup>158–160</sup> Treatment of **25** with silyl chlorides functionalizes N<sub>2</sub> to give iron silyldiazenido complexes **26**. Catalytic reduction of N<sub>2</sub> to ammonia can be achieved using KC<sub>8</sub> and [H(OEt)<sub>2</sub>]<sub>2</sub>BAR<sub>4</sub><sup>F</sup> catalyzed by **23** at –95 °C, with yields of up to 3.3 ± 1.1 equiv of NH<sub>3</sub> per iron.<sup>157</sup> Thus, the electron-rich CAAC ligands and the low coordination number are particularly effective for N<sub>2</sub> activation. Other Fe–N<sub>2</sub> complexes supported by chelating NHC ligand frameworks are also known (e.g., **27a–c**, Figure 11), but have not been studied as extensively.<sup>161–164</sup>



**Figure 9.** Structures and reactivity of synthetic iron-carbide complexes.



**Figure 10.** N<sub>2</sub> binding and functionalization using CAAC ligands. Ar = 2,6-<sup>i</sup>Pr<sub>2</sub>C<sub>6</sub>H<sub>3</sub>. R = Me, Et.



**27a**, L = N<sub>2</sub>; **27b**, L = C<sub>2</sub>H<sub>4</sub>; **27c**, L = PMe<sub>3</sub>

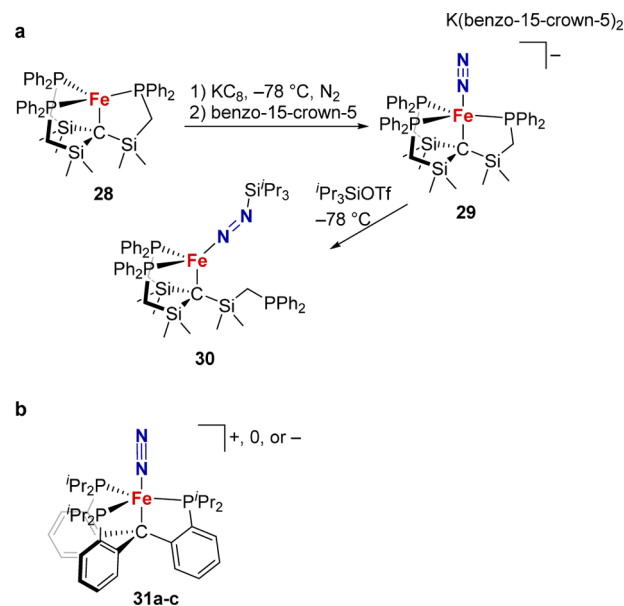
**Figure 11.** N<sub>2</sub> binding to Fe using NHC ligands.

Alkyl ligands have also been used as carbide mimics. In a systematic set of studies, Peters described trigonal pyramidal complexes of a tris(phosphine) platform with different alkyl ligands placed in one axial position (Figure 12). First, the Fe<sup>+</sup> complex **28** was reduced to give an Fe<sup>0</sup> complex with N<sub>2</sub> binding *trans* to the carbon ligand (**29**, Figure 12a).<sup>60</sup> Upon reduction and N<sub>2</sub> binding, the Fe–C distance in **28** (2.153(2) Å) elongated to 2.254(5) Å. Treatment of the complex with a silyl triflate at low temperature affords four-coordinate Fe diazenido complex **30**, with Fe–C = 2.116(1) Å. These show the great flexibility of the Fe–C bond, supporting model 1 for N<sub>2</sub> binding in the FeMoco (Figure 2).

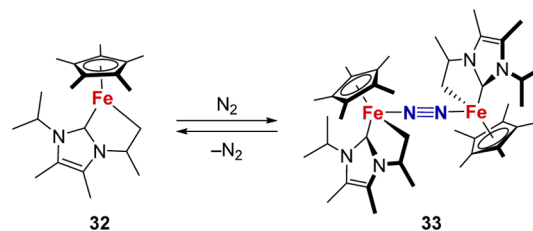
A related system with aryl linkers gave N<sub>2</sub> complexes having three different oxidation states (**31**, Figure 12b).<sup>61</sup> Fe–C bond lengthening from 2.081(3) Å in the cationic to 2.165(2) Å in the anionic complex again showed the flexibility of the Fe–C bond, and the anionic complex is capable of catalytic ammonia production (47 equiv/Fe).<sup>165</sup>

Ohki and co-workers reported a coordinatively unsaturated iron complex **32**, from solutions of which diiron–N<sub>2</sub> complex **33** crystallizes under N<sub>2</sub> atmosphere (Figure 13).<sup>166</sup> Complex **33** contains a Fe–N<sub>2</sub> moiety in a solely carbon-based ligand environment, with Cp\*, NHC, and an alkyl donor. The N<sub>2</sub> in **33** is held weakly, as indicated by reversion to **32** upon dissolving, and by a high N–N stretching frequency of 2126 cm<sup>-1</sup>.

Further examples of C-ligated Fe–N<sub>2</sub> complexes include bis(imino)pyridine-stabilized complexes with alkyl and aryl ligands<sup>167–169</sup> and also bis-cyclometalated CPC and CNC pincer supported complexes,<sup>170,171</sup> as well as N<sub>2</sub> complexes stabilized by η<sup>6</sup>-interactions with arenes.<sup>172,173</sup> In the long run,



**Figure 12.** Fe–N<sub>2</sub> complexes with *trans* carbon and N<sub>2</sub> ligands.



**Figure 13.** N<sub>2</sub> binding to Fe using multiple carbon-based ligands.

all of these complexes will add to our knowledge about the influence of donor power and geometry on N<sub>2</sub> activation by iron.

## 6. IRON–H COMPLEXES WITH SULFUR AND N<sub>2</sub> LIGANDS

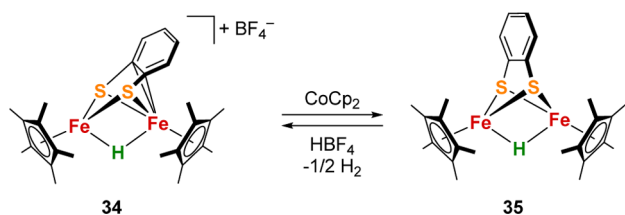
As described above, the E<sub>4</sub>H<sub>4</sub> state of nitrogenase has been shown by ENDOR spectroscopy to have two H atoms with direct bonds to iron (Fe–hydrides).<sup>86–89</sup> In principle, these hydrides could be either terminal (Fe–H) or bridging (Fe–H–Fe), and *ab initio* studies of a simplified model have evaluated the relative energies of these different binding modes.<sup>174</sup> The ENDOR spectra of E<sub>4</sub>H<sub>4</sub> show an intrinsic T tensor (coupling between the unpaired spin and the <sup>1</sup>H spin) that is rhombic.<sup>6,86</sup> Analogous ENDOR experiments on isolable Fe–H compounds with terminal hydrides showed an axial tensor, while bridging hydrides gave a rhombic tensor.<sup>6,175,176</sup> This combination of experiments validated the idea that the signals from the FeMoco-hydride (“E<sub>4</sub>”) are most consistent with bridging hydrides in E<sub>4</sub>H<sub>4</sub>.<sup>6</sup> However, the active form might have transient terminal hydrides, as is currently believed for hydrogenase enzymes based on synthetic modeling studies.<sup>177</sup>

Most isolated iron-hydride species have low-spin electronic configurations, whereas the weak-field environment of the iron atoms in the FeMoco is electronically different. This motivates the preparation of new iron-hydrides in weak-field ligand coordination environments, particularly those having sulfur-based ligands. We developed low-coordinate Fe(μ-H)<sub>2</sub>Fe dimers that have weak-field ligand environments, and these



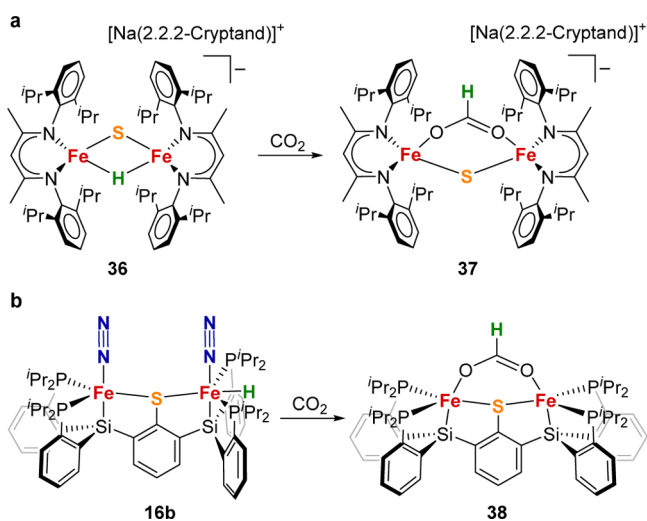
react with nitrogenase-relevant substrates such as alkynes, CO<sub>2</sub>, cyanide, and azide (but only react with N<sub>2</sub> upon irradiation).<sup>178–180</sup> Murray recently reported Fe<sub>3</sub>(μ-H)<sub>3</sub> clusters stabilized by a trinucleating β-diketiminato scaffold, which react with CO<sub>2</sub> but not other nitrogenase substrates.<sup>181</sup> However, none of the above iron-hydride species contained S donors.

Iron-hydride–thiolate complexes without CO or P ligands are rare.<sup>182,183</sup> Qu and co-workers recently reported diiron-hydrides in a sulfur- and carbon-rich environment, which are bridged by bis(thiolate) and stabilized by Cp\* ligands (34 and 35, Figure 14).<sup>183</sup>



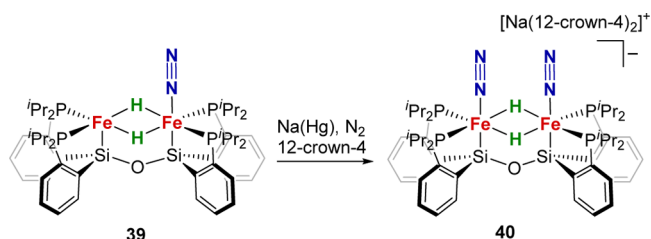
**Figure 14.** Bridging iron-hydrides in sulfur- and carbon-rich environment.

A recent report from our group introduced the sulfide and hydride bridged diiron complex 36 (Figure 15a), which models a potential coordination mode for hydrides in FeMoco as depicted in 4 (Figure 2 above).<sup>182</sup> Hydride complex 36 reacted with CO<sub>2</sub> to give bridging formate complex 37, which is relevant to CO<sub>2</sub> reduction by nitrogenase.<sup>184–186</sup> Complex 16b from above,<sup>120</sup> which has a terminal hydride (Figure 15b), also reduces CO<sub>2</sub> to give a thiolate bridged formate (38). Therefore, both bridging and terminal hydrides appear to be active in S-supported Fe–H complexes, at least for CO<sub>2</sub> reduction.



**Figure 15.** Iron-hydrides with sulfur ligands that reduce CO<sub>2</sub>.

Formation of N<sub>2</sub> complexes within an iron–sulfide–hydride framework is particularly relevant to the proposed reductive elimination of two hydrides from E<sub>4</sub>H<sub>4</sub>. Reduction of 36 by 2e<sup>−</sup> under N<sub>2</sub> resulted in the formation of a diiron(0)–N<sub>2</sub> complex.<sup>182</sup> Liberation of H<sub>2</sub> is observed, suggesting a parallel to the reductive elimination in the Thorneley–Lowe scheme. However, 36 has only one hydride and therefore cannot reductively eliminate H<sub>2</sub> in an intramolecular reaction. In



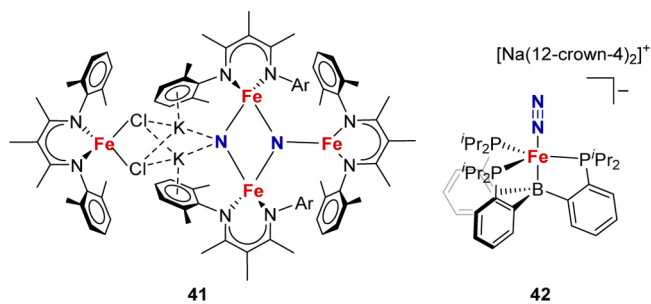
**Figure 16.** Binding of N<sub>2</sub> in the presence of bridging hydrides.

addition, the low yields of the N<sub>2</sub> complex and H<sub>2</sub> (~25%) make the significance of H<sub>2</sub> evolution in this system uncertain.

Studies of N<sub>2</sub> binding to Fe complexes with multiple hydrides are more relevant to N<sub>2</sub> binding from 4 (Figure 2). Though well-precedented with other metals,<sup>81,82</sup> such studies are rare with Fe complexes.<sup>187–189</sup> The Peters group recently reported the mixed-valent Fe<sup>2+</sup>(μ-H)<sub>2</sub>Fe<sup>+</sup> complex 40, which displays 10<sup>6</sup>-fold enhancement of N<sub>2</sub> binding affinity over Fe<sup>2+</sup>(μ-H)<sub>2</sub>Fe<sup>2+</sup> species 39 (Figure 16).<sup>119</sup> Treatment of 39 with excess KC<sub>8</sub> and [(Et<sub>2</sub>O)<sub>2</sub>H]BAR<sup>F</sup><sub>4</sub> resulted in production of 1.4 ± 0.5 mol equiv NH<sub>3</sub>. This is the first example of bridging hydrides in an Fe–N<sub>2</sub> complex, which is relevant to N<sub>2</sub> binding by E<sub>4</sub>H<sub>4</sub>. The authors suggest a model in which the role of hydrides may be to increase the ligand field at iron, stabilizing a low-spin electronic configuration that is preferred for N<sub>2</sub> binding. However, many iron–N<sub>2</sub> complexes with highly activated N<sub>2</sub> ligands have been observed in high-spin complexes of iron, so the need for low-spin Fe is not clear.<sup>65,158–160,190</sup> Overall, more studies are needed, particularly on S-supported systems, to draw firmer conclusions about the dependence of N<sub>2</sub> binding and H<sub>2</sub> elimination on the spin state of iron.

## 7. IRON–N<sub>x</sub>H<sub>y</sub> COMPLEXES WITH SULFUR LIGANDS

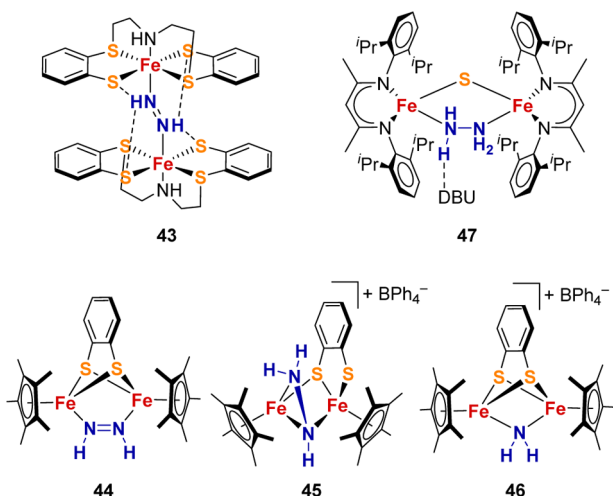
N<sub>2</sub> binding and reduction is now well established with iron complexes in P and N ligand environments. Recent highlights include cleavage of N<sub>2</sub> to two nitrides (41) and catalytic systems for conversion of N<sub>2</sub> to ammonia by mononuclear model complexes, the most successful being 42 (Figure 17).<sup>41,44,61,157,191,192,165</sup> Peters has shown that phosphine ligands provide excellent platforms for functionalization of N<sub>2</sub> on iron,<sup>60,193,194</sup> and enable characterization of potential N<sub>2</sub> reduction intermediates Fe–N<sub>x</sub>H<sub>y</sub>,<sup>98,99,165,194–196</sup> including systems that feature a coordinated hydride.<sup>40,42,43</sup> Ammonia production by tripodal trisphosphine ligands with an axial donor (Si, C, or B) increases in the order Si < C < B.<sup>165</sup> The higher degree of Fe–heteroatom bond flexibility in 42 could be crucial for stabilizing the variety of N<sub>2</sub> reduction intermediates in the catalytic cycle.<sup>61,192,193,197</sup> In a stoichiometric study using



**Figure 17.** Recent examples of N<sub>2</sub> activation on iron with N- and P-based supporting ligands.

the B-based ligand, an  $\text{Fe}\equiv\text{N}-\text{NH}_2$  intermediate was characterized that models the distal mechanism for  $\text{N}_2$  reduction (Figure 4).<sup>98</sup> Subsequent study with a Si-based ligand postulated a pathway for  $\text{N}_2$  reduction that includes  $\text{Fe}=\text{N}-\text{NH}_2$  and  $\text{Fe}-\text{NH}_2\text{NH}_2$  species.<sup>99</sup> However, sulfur-supported complexes are needed to more closely resemble the iron environment in the FeMoco.

Sellmann and co-workers characterized hydrazine, diazene, and  $\text{NH}_3$  complexes using multidentate thioether/thiolate ligands.<sup>198,199</sup> The *trans*- $\text{N}_2\text{H}_2$  ligand in complex 43 is bound to two iron centers and stabilized by hydrogen bonding interactions with S-ligands, a scenario that is feasible in the FeMoco (Figure 18). However, no  $\text{N}_2$  complex was accessible in this system. More generally, no reported system with S donors has yet been observed to bind  $\text{N}_2$  and to bind partially reduced  $\text{N}_x\text{H}_y$  species. Additionally, none of these S-based systems has been reported to perform  $\text{N}_2$  reduction, though some are capable of catalytic reduction of hydrazine.<sup>200,201</sup>



**Figure 18.**  $\text{Fe}-\text{N}_x\text{H}_y$  species in sulfur-rich environments. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

In a notable contribution, Qu and co-workers used a thiolate-bridged diiron system that binds *cis*-diazene (44), and this complex can be protonated to give  $\text{N}_2\text{H}_3$  species 45 (Figure 18).<sup>58</sup> Upon treatment with 2 equiv of a reducing reagent and protonation, 45 is transformed into the bridging amide complex 46. These reactions are relevant because transformation of  $\text{N}_2$  to ammonia might be mediated by at least two neighboring Fe atoms in the FeMoco through a series of  $\text{N}_x\text{H}_y$  bridged intermediates.<sup>66</sup> This  $\text{Cp}^*/\text{thiolate}$ -supported system is capable of hydrazine reduction as well.<sup>58</sup>

Binding of nitrogenase-relevant  $\text{N}_x\text{H}_y$  intermediates to Fe complexes with sulfides, rather than thiolates, is rare. Holland and co-workers reported a coordinatively unsaturated diiron sulfide species, to which  $\text{N}_2\text{H}_4$  and a substituted hydrazido ligand can bridge in a  $\mu-\eta^1:\eta^1$  fashion (47, Figure 18).<sup>202,203</sup> Complexes of substituted hydrazines have greater stability in this system,<sup>203</sup> and one mixed-valence system was studied using ENDOR for comparison of hyperfine parameters to nitrogenase intermediates.<sup>204</sup> However, this diiron sulfide system has not yet been observed to bind  $\text{N}_2$  without loss of sulfide. More model complexes are needed that have the combination of sulfur rich ligand environments,  $\text{N}_2$  binding, and the ability to stabilize various  $\text{Fe}-\text{N}_x\text{H}_y$  intermediates.

## 8. CONCLUSION AND OUTLOOK

At the turn of the century, synthetic modeling of nitrogenase was based on molybdenum, because of the demonstrated ability of molybdenum complexes to reduce  $\text{N}_2$  to ammonia.<sup>37</sup> However, emerging spectroscopy, crystallography, and biosynthesis of the enzyme has suggested iron binding of  $\text{N}_2$  instead. The broader acceptance of the iron hypothesis has crucially depended on advances in the understanding of synthetic iron complexes. Most notably, it has been shown that iron species are capable of reducing  $\text{N}_2$  to  $\text{NH}_3$ , including several cases with catalytic reduction of  $\text{N}_2$  noted above. Equally influential has been the realization that other hypothetical features of FeMoco intermediates, such as high-spin iron-hydrides, iron-diazenes, and iron-hydrazines, are feasible structures in the presence of sulfides and other sulfur-based donors. Spectroscopic studies on these species provide signatures for use in identifying intermediates, and their reactivity shows what reactions are reasonable to expect on the FeMoco.

Many of the initial advances in  $\text{N}_2$  reduction have been enabled by N- and P-based ligands, even though FeMoco iron sites use S and C donors. Thus, the next stage in nitrogenase modeling is to incorporate S and C donors into the model iron complexes. Although preparation of an exact model of the FeMoco would be an amazing synthetic achievement, perfect structural fidelity is not needed for deep insight. For example, analogous systems with and without S and C ligands enable us to understand the influence of these ligand types on the FeMoco reactivity and spectroscopy. Further, there is a need for systems that allow observation of the elementary steps that link  $\text{N}_x\text{H}_y$  species. These use PCET,<sup>83,205</sup> which has not yet been studied in the context of nitrogen reduction. The addition of single electrons and protons as part of the Thorneley–Lowe scheme suggests that PCET is crucial for nitrogenase catalysis, as noted above.<sup>6,7,24</sup> There have only been a few studies on PCET for any iron–sulfur clusters.<sup>64,206–208</sup> Studies on PCET to  $\text{N}_2$ ,  $\text{N}_2\text{H}_2$ ,  $\text{N}_2\text{H}_4$ , and other fragments of relevance to  $\text{N}_2$  reduction are needed,<sup>99,209</sup> because such reactions are involved in the part of the nitrogenase mechanism spanning the  $E_4$  to  $E_8$  levels. We also note that systems that engage in PCET often involve hydrogen bonds,<sup>210</sup> but to our knowledge there are not yet any examples of hydrogen bonding to an  $\text{N}_2$  unit in a transition-metal complex. Thus, this emerges as another synthetic challenge.

Another priority is the understanding of reversible  $\text{H}_2$  loss with  $\text{N}_2$  binding. Reductive elimination of  $\text{H}_2$  simultaneously generates an open site and two reducing equivalents on the metal, both of which contribute to  $\text{N}_2$  binding ability.<sup>81,82</sup> Interestingly, the available evidence on nitrogenase indicates that  $\text{H}_2$  loss, though reversible, occurs only upon addition of  $\text{N}_2$ . This observation suggests that the enzyme might generate an iron– $\text{H}_2$  complex that undergoes an associative ligand exchange reaction to bind  $\text{N}_2$ . This idea was also recently suggested by the kinetics of  $\text{H}_2$  loss upon photolyzing nitrogenase.<sup>88</sup> The reductive elimination of  $\text{H}_2$  provides an ideal way for the FeMoco to store reducing equivalents while leaving the iron sites in reasonable oxidation states. Though two electrons are “wasted” when  $\text{H}_2$  is lost, the need for nitrogen fixation makes this a worthwhile bargain of thermodynamics for kinetics. (*In vivo*, it is also likely that the “lost”  $\text{H}_2$  can be reabsorbed for recovery of some of the energy.) Synthetic iron compounds that exchange  $\text{H}_2$  and  $\text{N}_2$  have been studied in phosphine-containing systems,<sup>211,212</sup> and



should be extended to sulfur-containing environments. Also, a biomimetic strategy of H<sub>2</sub> replacement may enable chemists to forego the use of very strong acids and reducing agents during the catalytic reduction of N<sub>2</sub>.<sup>165</sup> Artificial systems that use light could provide an attractive means for driving the kinetically challenging dinitrogen reduction, and recently, N<sub>2</sub> reduction driven by visible light has been observed from nanoparticle-enzyme biohybrids and from iron-sulfide gels.<sup>213–215</sup>

This Perspective has highlighted these and other areas in which synthetic chemistry will continue to enrich our understanding of the fascinating process of reducing N<sub>2</sub>.

## AUTHOR INFORMATION

### Corresponding Author

\*patrick.holland@yale.edu

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

Nitrogen research in the Holland laboratory has been generously supported by the National Institutes of Health (GM065313).

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