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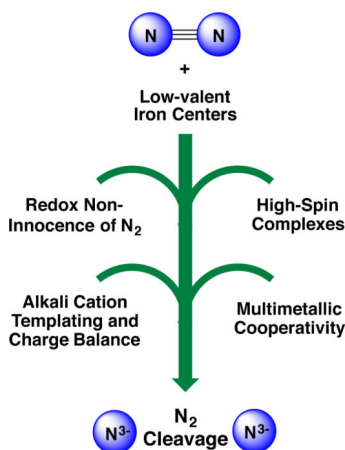
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## Dinitrogen Binding and Cleavage by Multinuclear Iron Complexes

SEAN F. MCWILLIAMS and PATRICK L. HOLLAND\*

Department of Chemistry, Yale University, New Haven, CT 06511

### Graphical Abstract



### Introduction: How Does the FeMoco Work?

Nitrogenase enzymes provide the world's largest input of fixed nitrogen, because they have the amazing ability to catalytically reduce dinitrogen to ammonia under ambient conditions of room temperature and 0.8 atmospheres of  $N_2$ . Though the activation barrier for this room temperature catalytic reaction is low compared to that for the high-temperature Haber-Bosch process, the biological reaction is driven thermodynamically by the hydrolysis of at least 16 molar equivalents of ATP per molecule of  $N_2$ .<sup>1</sup> Kinetic studies on the molybdenum-iron enzyme, and the fact that the biological reductant transfers one electron at a time, suggest that there are at least eight intermediates during the catalytic cycle, which strains the ability of bioinorganic chemists to establish the mechanism in detail.<sup>2</sup> In addition, the structure of the FeMoco (Figure 1) consists of eight metal ions, whose oxidation states are still the subject of debate.<sup>3</sup> As a result, there are great opportunities and great challenges in understanding the fundamental chemistry that underlies biological dinitrogen reduction by nitrogenases.

A number of studies over the last fifteen years have led most chemists in the field to consider the most likely substrate binding site to be one or more of the central iron atoms, on the side facing His-195 and Val-70 of the  $\alpha$  subunit. This idea was originally supported by the

\*Corresponding Author [patrick.holland@yale.edu](mailto:patrick.holland@yale.edu).

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activity of enzyme variants derived from mutation of these residues and from ENDOR studies of trapped intermediates,<sup>4</sup> and it has recently gained additional support in the crystal structure of a CO-inhibited state in which a sulfide on the same face is replaced by a bridging CO.<sup>5</sup> Focus on the central part of the FeMoco is also bolstered by the identification of a carbide that bridges the six belt iron atoms. The observation of a carbide in an enzyme is unique to nitrogenase, and extremely surprising. However, the identification of the central atom as C, initially proposed on the basis of X-ray emission,<sup>6,7</sup> crystallographic and ESEEM measurements,<sup>8</sup> has now been firmly established through specific <sup>14</sup>C labeling of the carbide precursor and following the radiolabel through a series of biosynthetic precursors into the active protein.<sup>9</sup> The presence of such an unusual structural feature, and the fact that there is cellular machinery devoted to installation of C into the FeMoco, implies that the carbide plays a crucial role in catalysis.

Figure 2 shows two hypothetical roles for the carbide during reduction of the cluster to the reactive, reduced form. One possibility (Figure 2a) proposes “hemilabile” bonding between the Fe and C atoms. In this hypothesis, flexing of the core to break or weaken a Fe-C bond would play a key role in N<sub>2</sub> binding and further reduction steps. Peters has established that Fe-C distances can vary widely in a series of N<sub>2</sub>-binding compounds capable of reducing N<sub>2</sub> to NH<sub>3</sub>, which supports this idea.<sup>10</sup> In a second possibility (Figure 2b), a Fe-S bond is cleaved and the Fe-C bonds remain intact. This possibility is supported by the observation of similar metal-metal distances (via EXAFS) in the FeMoco resting state and intermediates,<sup>11</sup> by Fe-S cleavage in smaller Fe-S clusters upon protonation,<sup>12</sup> and by the crystallographic observation of sulfide displacement from the FeMoco by CO.<sup>5</sup> Each hypothetical mechanism gives a partially or completely open coordination site at which N<sub>2</sub> could form a bond to an Fe atom.

The resulting Fe-N<sub>2</sub> compounds are very unusual from the perspective of coordination chemistry.<sup>13</sup> At the outset of our Fe-N<sub>2</sub> research in 2000, there were no examples of Fe-N<sub>2</sub> compounds in which the coordination number was less than five, no examples of Fe-N<sub>2</sub> complexes with paramagnetic and/or high-spin metals, no Fe-N<sub>2</sub> complexes in which the N-N bond was substantially weakened as judged by crystallography or vibrational spectroscopy, and no examples of Fe complexes that gave more than about 10% yield of the expected 2 NH<sub>4</sub><sup>+</sup> from reduction/protonation of N<sub>2</sub>. The lack of compounds of these types hindered efforts to determine which hypothetical intermediates are reasonable, and whether an iron-based mechanism was reasonable at all. We anticipated that new Fe-N<sub>2</sub> complexes with Fe in environments more reminiscent of the enzyme could provide fundamental insights. This Account focuses on the evolution of my group's research in this area. We also recommend reviews that cover a broader range of metals for N<sub>2</sub> reduction.<sup>14,15,16</sup>

## Low Coordination Numbers at Iron Enhance N-N Bond Weakening

Low-spin N<sub>2</sub> complexes of iron(0) and iron(II) in the literature have relatively weak backbonding that leads to high N-N stretching frequencies and short N-N triple bonds. This generalization holds for both terminal and end-on/end-on bridging complexes of N<sub>2</sub>.<sup>13</sup> However, when we prepared three-coordinate, formally diiron(I) complexes of N<sub>2</sub>, we discovered that they have much lower stretching frequencies (by more than 200 cm<sup>-1</sup>) and

longer N-N bonds (by more than 0.07 Å).<sup>17</sup> The low coordination number in these complexes is enforced by bulky β-diketiminato ligands (Figure 3).

Studies on the electronic structure of the complex  $L^{\text{Me,iPr}}\text{FeNNFe}L^{\text{Me,iPr}}$  using ligand-field calculations and Mössbauer spectroscopy (in collaboration with Sebastian Stoian, Emile Bominaar and Eckard Münck) suggested a resonance structure that has two iron(II) ions and a bridging  $\text{N}_2^{2-}$  ligand.<sup>18</sup> These two  $S = 2$   $\text{Fe}^{2+}$  sites can each couple antiferromagnetically with the  $S = 1$   $\text{N}_2^{2-}$  group to give a total  $S = 3$ , consistent with Mössbauer and magnetic susceptibility data.<sup>19</sup> We have also pursued density functional theory computations (BP86/TZVP) on this molecule in the septet ( $S = 3$ ) ground state, which give excellent agreement (within 0.02 Å) with metrical parameters within the core and also excellent agreement with the Mössbauer parameters (exp,  $\delta = 0.62$  mm/s,  $|E_Q| = 1.41$  mm/s; calcd with TPSSh/TZVP at the BP86-optimized geometry,  $\delta = 0.56$  mm/s,  $|E_Q| = 1.27$  mm/s). These validated calculations show Mulliken spin populations of +3.2 on each iron atom and -0.6 on the  $\text{N}_2$  unit. Analysis of the ground state using broken-symmetry methods shows that two of the  $\alpha/\beta$  pairs have an overlap ( $S_{\alpha\beta}$ ) of only 89%, and the  $\alpha$  spin is more localized on the iron atoms while the  $\beta$  spin is more localized on the  $\text{N}_2$   $\pi^*$  orbital (Figure 4). These “correlated pairs” are characteristic of redox non-innocent ligands, in agreement with the triplet  $\text{N}_2^{2-}$  interpretation.

Other iron species with lower coordination numbers are also capable of weakening N-N bonds, as shown in bridging diiron(I)- $\text{N}_2$  complexes reported by Peters and Arnold.<sup>20,21</sup> We have also shown the reduction of  $L^{\text{Me,iPr}}\text{FeNNFe}L^{\text{Me,iPr}}$  and its analogue  $L^{\text{tBu,iPr}}\text{FeNNFe}L^{\text{tBu,iPr}}$  by two electrons to yield complexes of the type  $\text{M}_2[\text{LFeNNFeL}]$  ( $\text{M} = \text{Na}, \text{K}$ ).<sup>17</sup> Preliminary DFT calculations indicate greater spin population on  $\text{N}_2$  (-1.0), which is consistent with the lower observed stretching frequency ( $\nu_{\text{NN}} = 1589\text{-}1625$   $\text{cm}^{-1}$ ). Therefore, though these complexes are formally diiron(0), it is likely that they are best viewed as  $\text{Fe}^{1+}\text{-N}_2^{2-}\text{-Fe}^{1+}$ .

Despite the negative charge on the  $\text{N}_2$  unit, reactions at  $\text{N}_2$  were not evident in the diketiminato-supported complexes. The most characteristic reaction was loss of  $\text{N}_2$ , with transfer of electrons back to the iron, and coordination of an additional (typically  $\pi$ -acceptor) ligand or reaction with an oxidant.<sup>17b</sup> Binding of alkenes, alkynes, and phosphine gave iron(I) complexes in thermodynamically favorable reactions.<sup>22</sup> Treatment of the  $\text{N}_2$  complexes with acids yielded no detectable ammonia. Unreported studies even used “tethered” acids: for example, since pyridine coordinates to  $L^{\text{tBu,iPr}}\text{FeNNFe}L^{\text{tBu,iPr}}$ , we hoped that 2-hydroxypyridine would suspend an acidic proton immediately above the  $\text{N}_2$  unit. However, this reaction yielded only the complex shown in Figure 5. Thus, the ability of the low-coordinate environment to “push” electron density into the  $\pi^*$  orbitals of  $\text{N}_2$  did not translate into the desired reactions of coordinated  $\text{N}_2$ .

## Cooperation Between Three Iron Atoms Enables N-N Cleavage to Nitrides

At this point, we hypothesized that the steric bulk of the ligands prevents  $\text{N}_2$  from reacting with acids and other potential electrophiles. Therefore, we decreased the size of the arene substituents from isopropyl to methyl in the new ligand  $L^{\text{Me}3}$  (Figure 6). Since our

successful synthesis of  $L^{\text{Me,iPr}}\text{FeNNFe}L^{\text{Me,iPr}}$  in previous work used reduction of iron(II) chloride complex  $[L^{\text{Me,iPr}}\text{Fe}(\mu\text{-Cl})_2]$  with 2 equiv of potassium graphite ( $\text{KC}_8$ ), we used the analogous reaction of  $[L^{\text{Me}^3}\text{Fe}(\mu\text{-Cl})_2]$  with 2 equiv of  $\text{KC}_8$ . This reaction yielded a very different product, shown in Figure 6.<sup>23</sup>

This product has four iron atoms, two  $\text{K}^+$  ions, two  $\text{Cl}^-$  ions, and two nitrides; isotope-labeling studies show that the nitrides come from  $\text{N}_2$ . The complete reduction of  $\text{N}_2$  to two  $\text{N}^{3-}$  ions requires six electrons, and this reduction half-reaction can be balanced by the net oxidation of four  $\text{Fe}^{1+}$  to two  $\text{Fe}^{2+}$  and two  $\text{Fe}^{3+}$ , as indicated in Scheme 1b. These oxidation states agree with charge counting within the core, and with the results of Mössbauer spectra from collaborator Eckhard Bill.

Though  $\text{K}^+$  is incorporated into the product, the complete reduction of  $\text{N}_2$  is not specific to potassium-containing reductants like  $\text{KC}_8$ . Interestingly,  $\text{RbC}_8$  gives an analogous product, in which  $\text{Rb}^+$  ions occupy the  $\text{K}^+$  positions.<sup>24</sup> Powdered sodium metal effects a similar transformation with full N-N cleavage, but the product has only one  $\text{Na}^+$  ion, and has lost one iron atom (presumably because  $\text{Na}^+$  is too small to bridge;  $[L^{\text{Me}^3}\text{Fe}(\mu\text{-Cl})_2]$  is observed as a byproduct that accounts for loss of this iron(II) ion).<sup>24</sup> Mössbauer spectra of the three systems (Na, K, Rb) are similar, but the spectrum of the Na complex is missing a signal with parameters characteristic of tetrahedral high-spin iron(II), as expected for loss of the dangling  $\text{Fe}^{2+}$ .

An important implication from these results is that  $\text{N}_2$  reduction is facilitated by cooperation between reduced iron species. Since three iron atoms are bound to the remnants of  $\text{N}_2$ , our collaborators Travis Figg and Thomas Cundari explored potential pathways for three-iron  $\text{N}_2$  cleavage, using density functional calculations on simplified models having no diketimate substituents ( $L^{\text{trunc}}$ , Figure 7).<sup>25</sup>

These calculations showed that three diketimate-bound iron(I) ions can interact with  $\text{N}_2$  in both end-on/end-on/side-on (EES) and end-on/side-on/side-on (ESS) binding modes that are connected by low activation barriers (one iron atom revolves partway around the core). The ESS binding mode can easily cleave the N-N bond without major geometric distortions, but the N-N cleaved product is not thermodynamically stable unless the negative charge on the two-coordinate nitride is stabilized by a positively charged ion like  $\text{K}^+$ . N-N cleavage also requires an “extra” electron beyond those provided by these three iron(I) ions, which is provided either by the dangling iron atom or by the sacrificial iron in the Na reaction. These studies suggested a thermodynamically and kinetically reasonable pathway to N-N cleavage at a triiron site: initial formation of an  $L^{\text{Me}^3}\text{FeNNFe}L^{\text{Me}^3}$  complex (analogous to those with bulkier ligands) is followed by coordination of a third iron(I) species in a side-on mode. The EES complex is only accessible with relatively small diketimate supporting ligands for steric reasons, explaining why N-N cleavage was not observed with  $L^{\text{tBu,iPr}}$  and  $L^{\text{Me,iPr}}$ . After rotation to the ESS mode, reduction by a fourth iron(I) enables N-N cleavage to the final product. In this mechanism, a likely role for the alkali metal is to stabilize the N-N cleaved product: thus it is a fundamentally thermodynamic rather than kinetic explanation for the alkali metal effect.

## Alkali Metals as Templating Elements for Multimetallic Reactions

It is also important to consider kinetic influences, in which alkali metals could facilitate the molecules achieving the correct transition state for the reaction. An important kinetic effect was suggested by our parallel reactions of  $[\text{L}^{\text{Me}_3}\text{Fe}(\mu\text{-Cl})]_2$  with cesium graphite ( $\text{CsC}_8$ ). Even though it should be an equally strong reductant as  $\text{KC}_8$  and  $\text{RbC}_8$ , use of 2 equiv or 4 equiv of  $\text{CsC}_8$  per iron(II) dimer gave products with no N-N cleavage.<sup>24</sup> A key product (Figure 8) had three end-on/end-on bridging  $\text{N}_2$  units and no nitrides. Subsequently, we learned that 4 equiv of  $\text{KC}_8$  or  $\text{RbC}_8$  give analogous triangular products. (The N-N cleaved products described above are not intermediates along the way to the triangular products; the additional reductant apparently reacts with an intermediate prior to N-N cleavage.) This significant observation indicates that more reducing agent does not necessarily result in a greater degree of N-N reduction, and that thermodynamic driving force is not the main determinant of reaction outcome. Rather, we surmised that different alkali metal cations, which coexist with iron(I) species in the unobserved intermediates, can “steer” the iron ions into favorable conformations for different reactions.

The main way that alkali metal cations can favor different conformations is through cation- $\pi$  interactions involving the supporting ligand, which we have observed in *all* anionic complexes of iron-diketiminato systems (except those where crown ethers were used to sequester these ions). In the formally diiron(0)- $\text{N}_2$  complexes described above, the  $\text{K}^+$  and  $\text{Na}^+$  ions not only coordinate to the aromatic rings, but also to the bridging  $\text{N}_2$  ligand. We have also observed the first iron(I) sulfide complexes, which benefit from  $\text{Na}^+$  or  $\text{K}^+$  binding to the aromatic rings and to the bridging  $\text{S}^{2-}$  ion.<sup>26</sup> The ability to stabilize highly reduced species is thermodynamically facilitated by the presence of positive charge near the reduced core.

However, we have also gained evidence that cation- $\pi$  interactions can influence the barriers for  $\text{N}_2$ -binding reactions in a way that controls reactivity. For example, unusual iron(I) phenoxide complexes  $[\text{KL}^{\text{tBu},\text{iPr}}\text{FeOPh}]_{1,2}$  react with  $\text{N}_2$  *only* when  $\text{K}^+$ -arene interactions hold the compound together in a dimeric form, with a geometry that is well-adapted to give bridging  $\text{N}_2$ .<sup>27</sup> Computational studies supported the idea that initial Fe- $\text{N}_2$  binding can happen equally well in both monometallic and bimetallic complexes, but the bimetallic complexes facilitate the bridging of  $\text{N}_2$  to give a more stable form. Thus, the bimetallic complexes, held together by cation- $\pi$  interactions, are more active toward cooperative reactions.

Extending this concept, we hypothesize that in the N-N bond cleaving reaction, either larger cations (in the case of  $\text{Cs}^+$ ) or more cations (in the complexes from over-reduction) give a geometry that holds Fe atoms too far apart to achieve the necessary  $\text{Fe}_3(\text{N}_2)$  intermediate, whereas the correct size *and* number of  $\text{Na}^+$ ,  $\text{K}^+$ , or  $\text{Rb}^+$  ions can steer the iron atoms into the correct relative orientation.<sup>24</sup> This hypothesis is difficult to test, because the key intermediates have not been isolated, and because there are many possible orientations of cation- $\pi$  interactions that would be difficult to evaluate computationally. However, this idea does suggest that the incorporation of alkali metal cations might be useful for a broad range

of reactions in which multimetallic cooperativity is useful, for example multielectron redox reactions such as water oxidation/O<sub>2</sub> reduction and CO<sub>2</sub> reduction.

This approach complements the strategy of using covalently-linked multinucleating ligands. For example, trimetallic complexes by Murray have been shown to bind N<sub>2</sub> at a tricopper(I) species, and also to cleave N<sub>2</sub> using a presumed triiron(I) intermediate.<sup>28,29</sup> Limberg has also prepared dinucleating diketiminate ligands and their complexes.<sup>30</sup> Multinucleating ligands from Betley have given multimetallic sites that reduce small molecules.<sup>31,32,33</sup> We anticipate that the use of alkali metals will be an easy way to reversibly steer metal atoms into close proximity for multimetallic reactions, without the synthesis of complicated ligands.

## Summary and Additional Perspectives on the Future

The fundamental work described above has led to important concepts, such as the use of low coordination numbers and a high-spin electronic configuration for N<sub>2</sub> weakening, involvement of several metal ions for easier activation of strong bonds, and manipulation of geometries and charge buildup with alkali metal cations. In order for these coordination chemistry concepts to be applied to the challenges of the FeMoco mechanism, several barriers must be overcome.

One challenge is that very strong reducing agents must be replaced by less aggressive reductants. Our research shows that alkali metal cations give more positive redox potentials, which could be useful for enabling milder reduction. Note that the FeMoco in nitrogenase is surrounded by a number of positively charged amino acid residues, including a His and Arg near the active four-iron face, which might play an analogous role. Another potential route to reduced metal-N<sub>2</sub> species without a strong reductant is by reductive elimination of hydrides as H<sub>2</sub>.<sup>34</sup> We have demonstrated this method of N<sub>2</sub> binding in both cobalt<sup>35</sup> and iron<sup>36</sup> complexes, and it has been proposed in the FeMoco based on H/D labeling and ENDOR studies.<sup>37</sup> It may also be possible to reduce metal-N<sub>2</sub> species at less negative potentials by coupling N<sub>2</sub> reduction with N<sub>2</sub> protonation. The study of metal-oxo intermediates has taught us that the ability to oxidize a substrate can be facilitated by increasing the basicity of the site that accepts a proton.<sup>38</sup> Likewise, the ability to reduce a complex can in principle be eased by providing a proton simultaneously (proton-coupled electron transfer or PCET).<sup>39</sup> PCET often has stringent geometric constraints, and geometric control is also necessary to make sure that protons and electrons go to N<sub>2</sub> reduction rather than H<sub>2</sub> formation.

Finally, the nature of the supporting ligands has not yet been evaluated in enough detail. The anionic nitrogen atoms in a diketiminate are weak-field  $\pi$ -donors like the biological sulfides, but the best supporting ligand would have S-based donors. Coordinatively unsaturated complexes rarely have thiolates or sulfides because of the tendency of sulfur to bridge between metals, forming clusters that fill any open coordination sites. As a result, the only published S-containing Fe-N<sub>2</sub> complexes have thioethers and non-S donors.<sup>40</sup> However, the FeMoco belt iron atoms have only S and C coordination, and nothing is known about Fe-N<sub>2</sub> binding in such an environment.

Overall, we hope that the perspectives above serve as encouragement for the study of more complexes with cooperative reactions, both between multiple transition metals and between transition metals and main-group metals, for the activation of strong bonds. The presence of clusters at some of the most potent bond-breaking and bond-forming enzymes (nitrogenase, photosystem II, methane monooxygenase, hydrogenase, CO dehydrogenase) is evidence that natural systems have benefitted from this strategy. Since there are so many permutations of metals and reactions, it is clear that the sky (with all of its N<sub>2</sub>) is the limit!

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## Biography

**Sean F. McWilliams** received a B.S. in chemistry with highest honors from Temple University in 2013. He is currently pursuing graduate studies with Patrick L. Holland at Yale University, focusing on fundamental iron-dinitrogen chemistry relating to nitrogenase and the Haber-Bosch process.

**Patrick L. Holland** received an A.B. in chemistry with high honors from Princeton University, and a Ph.D. from the University of California at Berkeley working on organometallic chemistry with Richard Andersen and Robert Bergman. After postdoctoral work with William Tolman at the University of Minnesota, his independent research has addressed mimics of several iron-containing metalloenzymes, particularly nitrogenases. Prof. Holland was on the faculty at the University of Rochester from 2000-2013, and he is now Professor of Chemistry at Yale University.

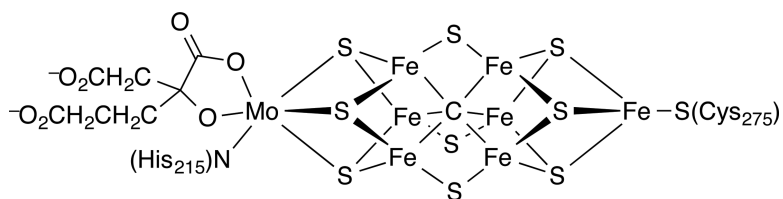
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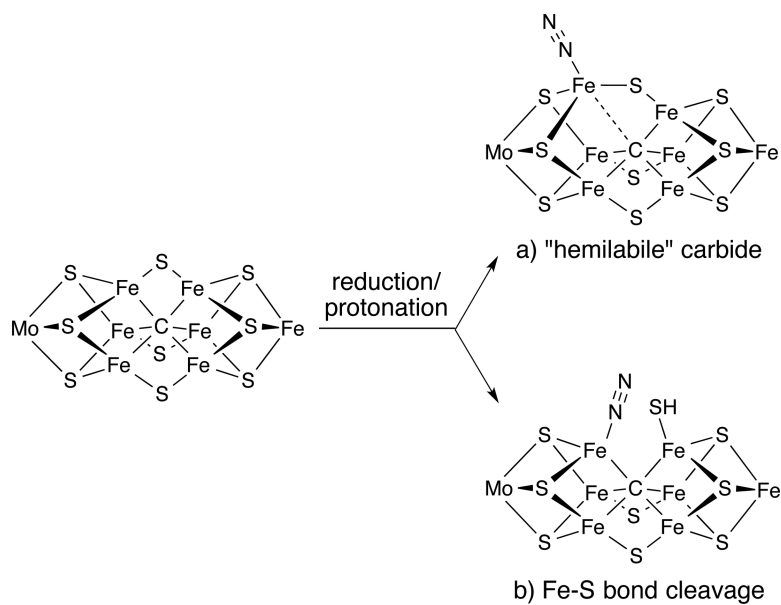
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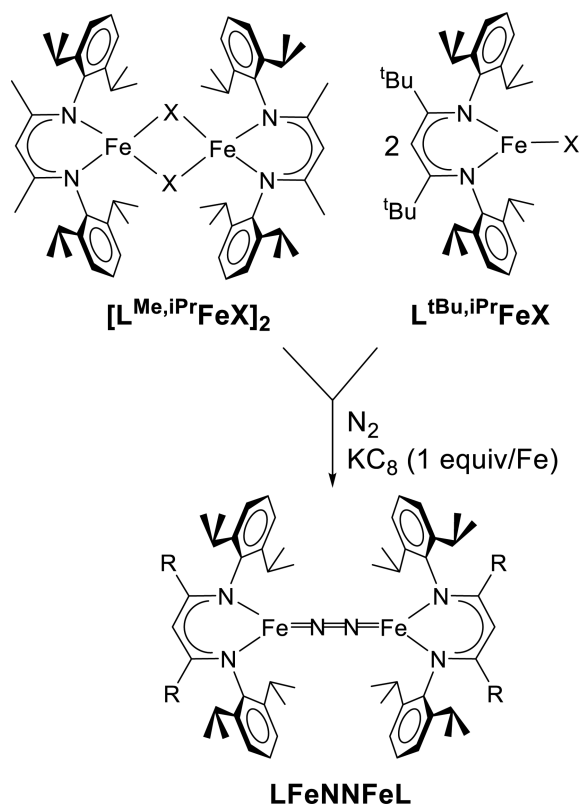
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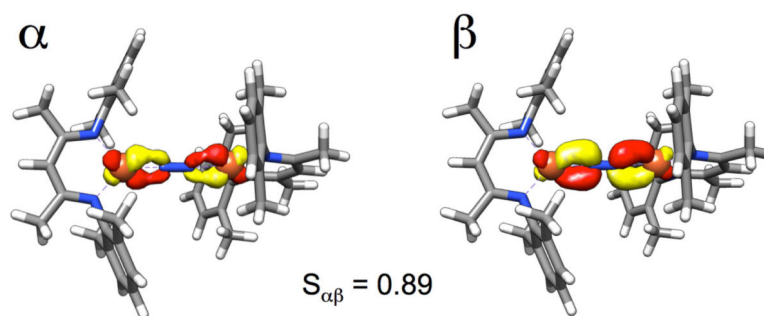
**Figure 1.** Structure of iron-molybdenum cofactor (FeMoco) of nitrogenase in its resting state.<sup>8</sup>



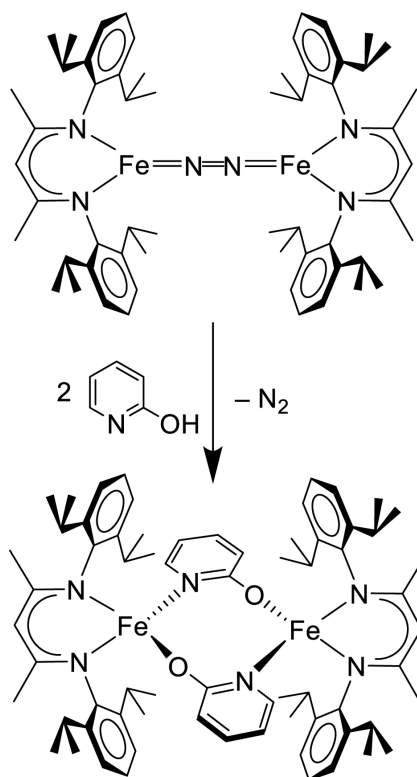
**Figure 2.** Two hypothetical activation modes in FeMoco: a) Fe-C weakening or cleavage (indicated by a dashed line), b) Fe-S cleavage (indicated with one possible product after protonation).



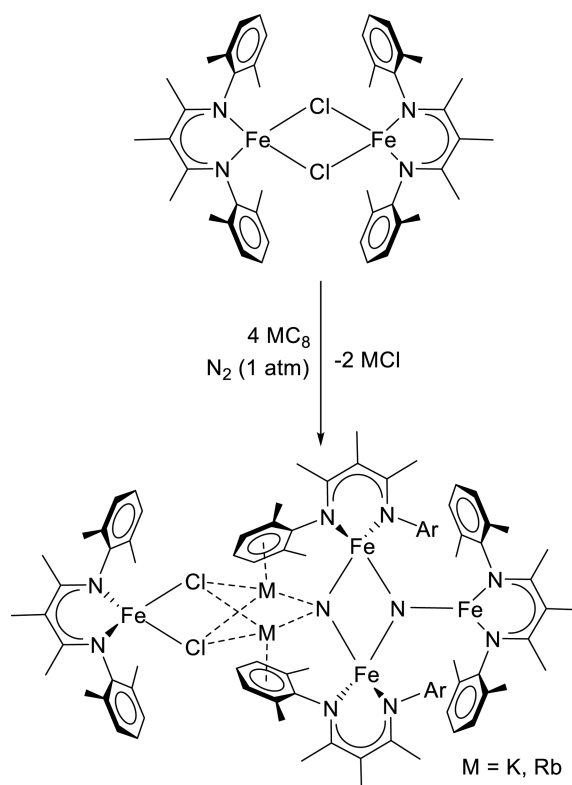
**Figure 3.**  $\beta$ -Diketiminato ligands stabilize low-coordinate iron complexes ( $\text{X} = \text{Cl}, \text{Br}$ ), which react with  $\text{N}_2$  under reducing conditions to give products with weakened  $\text{N}_2$ .



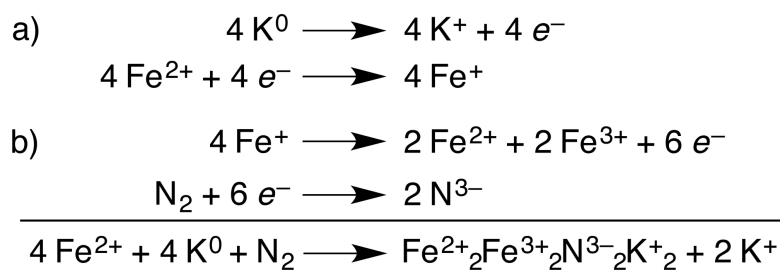
**Figure 4.** A key “correlated pair” of orbitals from broken-symmetry calculations on  $L^{Me,iPr}FeNNFeL^{Me,iPr}$ , showing that there is  $\beta$  spin more localized on the  $\pi^*$  orbital of  $N_2$ . ( $S_{\alpha\beta}$  is the value of the overlap integral between the  $\alpha$  and  $\beta$  orbitals.) This is consistent with antiferromagnetic coupling of an  $N_2^{2-}$  bridge to two  $Fe^{2+}$  ions.



**Figure 5.** Reaction of  $L^{\text{Me,iPr}}\text{FeNNFe}L^{\text{Me,iPr}}$  with 2-hydroxypyridine leads not to protonation by the pendent acid, but gives  $\text{N}_2$  displacement instead. Reduction of  $2 \text{H}^+$  to form  $\text{H}_2$  is assumed to account for the net oxidation from  $2 \text{Fe(I)}$  to  $2 \text{Fe(II)}$ .

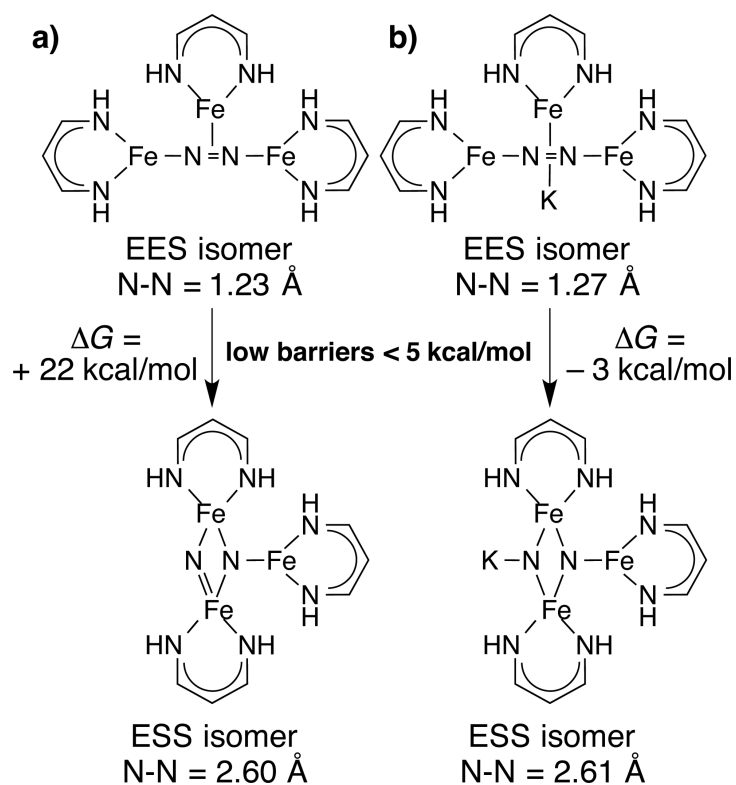


**Figure 6.** A smaller  $\beta$ -diketiminato,  $L^{\text{Me}_3}$ , gives a four-iron product where the N-N triple bond has been cleaved to form two nitrides.

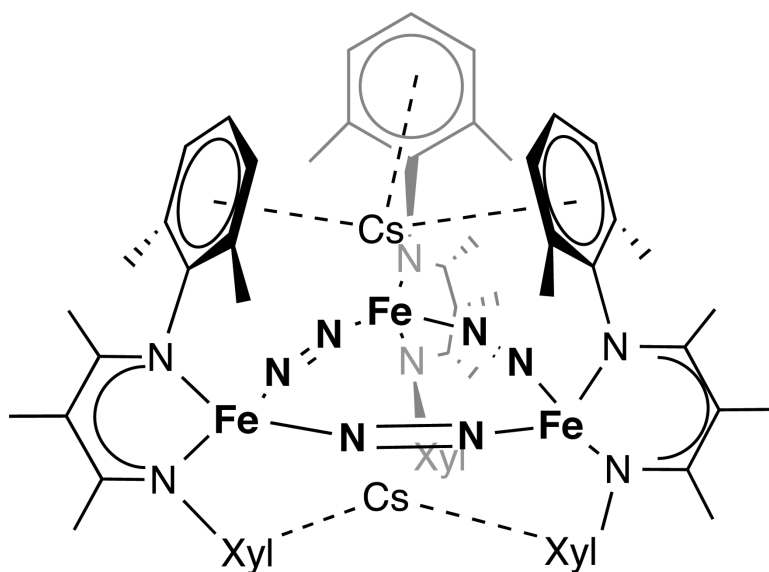
**Scheme 1.**

Series of half-reactions that lead to the bis-nitride product: a) the reduction of iron to the reactive iron(I) form, and b) the cleavage of N<sub>2</sub>. Two molar equivalents of KCl are also lost in the second reaction; these are represented as “2 K<sup>+</sup>” in the overall redox reaction.





**Figure 7.** Computation of the energy barrier for the conversion from end-on/end-on/side-on (EES) geometry to the end-on/side-on/side-on (ESS) geometry using truncated ligands. a) Computed barrier in the absence of potassium. b) Computed barrier in the presence of potassium. These results indicate that the addition of an electron and a potassium ion stabilizes the N-N cleaved isomer substantially, favoring the rearrangement of the core.



**Figure 8.** The product of reduction with 4 equiv of CsC<sub>8</sub> is Cs [L<sup>Me3</sup>Fe(μ-N<sub>2</sub>)]<sub>3</sub>, which has intact N-N bonds. This shows that more reducing conditions do not necessarily lead to N-N cleavage, and implies a kinetic influence on N<sub>2</sub> activation.