# The mysterious central atom in the nitrogenase FeMo cofactor

Kevin Hwang Literature Seminar December 6, 2011

The reduction of dinitrogen remains one of the biggest challenges in chemistry today. While industrial-scale production of ammonia has been made possible by the Haber-Bosch process, this process requires harsh conditions, with very high temperatures and pressures necessary in order to break the extremely robust N-N triple bond. In contrast, certain bacteria have evolved enzymes capable of catalytically reducing nitrogen to ammonia under ambient conditions (Eq. 1), a feat thus far unmatched by human ingenuity.[1]

$$N_2 + 8H^+ + 8e^- + 16MgATP \longrightarrow 2NH_3 + H_2 + 16MgADP + 16P_i \quad (1)$$

As a result, substantial effort has been applied towards understanding the mechanism of bacterial nitrogenases, and in particular one of the most studied is the molybdenum-containing nitrogenase (Mo-nitrogenase).[2] This review will cover recent work elucidating the structure of the iron-molybdenum cofactor, which in Mo-nitrogenase is believed to be the site of nitrogen binding and reduction.

The structure of nitrogenase from *Azotobacter vinelandii* was first reported in 1992 by Kim and Rees.[3] From this structure, the overall organization of nitrogenase was described. Nitrogenase contains two proteins - an iron-containing protein, with a [4Fe:4S] cluster used for electron transfer; and an iron-molybdenum protein, which contains a [8Fe:7S] cluster (the P cluster), also used for electron transfer, and an iron-molybdenum cluster known alternatively as the M cluster or the iron-molybdenum cofactor (FeMoco). The assigned structure contained a unique arrangement of seven iron atoms and one molybdenum atom, bridged by sulfur atoms, surrounding a cavity with diameter approximately 4Å (Figure 1).[3] Investigation of MoFe-deficient variants of nitrogenase have suggested that this cluster is the site of nitrogen fixation, and consequently this cluster has been the subject of a great deal of attention.[4]

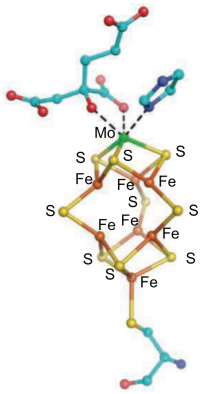


**Figure 1.** FeMoco structure (1992).[2]

In 2002, Rees and coworkers reported a refinement of nitrogenase to 1.16 Å resolution, which enabled the detection of electron density within the FeMoco cavity. The observed electron density was assigned to a light atom, most plausibly carbon, nitrogen, or oxygen. From the observed electron density alone, the central atom could not be conclusively identified, but on the basis of a resolution-dependent electron density profile the atom was tentatively assigned as nitrogen.[5] While this exciting discovery suggested potential catalytic significance for the FeMoco cavity, subsequent experiments led to somewhat contradictory results. Pulsed EPR (ENDOR and ESEEM) studies by Hoffman *et al.* initially suggested that the central atom could not result from exchange with environmental $N_2$,[6] and in later work further suggested that none of the observed nitrogen signals occurred from the FeMoco cluster.[7] Later, a combination of C-13 and N-15 isotope labeling, pulsed EPR and DFT calculations failed to detect any strongly coupled signals, indicating either that the central atom of FeMoco is neither carbon nor nitrogen, or that it is magnetically decoupled from the rest of the cluster.[8] However, DFT calculations performed

by Dance suggested that low spin density on the central atom might explain the apparent negative results obtained in prior ESEEM and ENDOR experiments.[9]

Recently, DeBeer and coworkers described the use of valence-to-core X-ray emission spectroscopy (V2C XES) to successfully identify light atoms bound to iron atoms.[10] By the use of XES and DFT calculations, the observed spectrum from FeMoco was fit most closely to a structure containing a central carbon atom (Figure 2).[11] Simultaneously, a report from Einsle and Rees reported a re-refinement of FeMoco at 1.0 Å resolution, in which the central atom was more clearly a carbon atom, as well as an improved isotopic labeling procedure that enabled detection of a central carbon signal in the ESEEM spectrum of C-13 labeled FeMoco.[12] With this confluence of experimental data, there is now strong evidence to suggest the central atom in the FeMoco cluster is carbon.

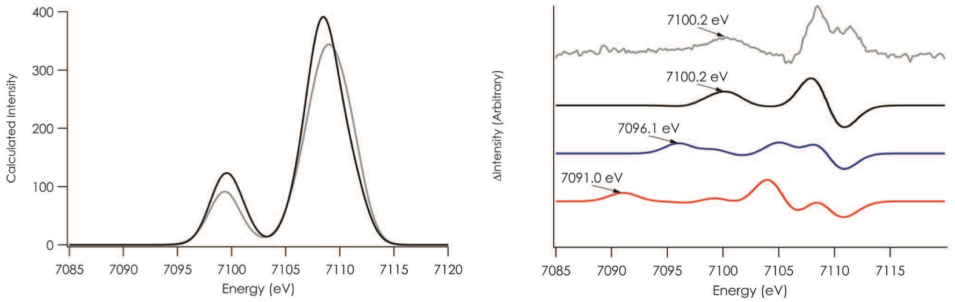


**Figure 2**. (Left) Calculated XES spectrum of carbon-containing (black) and empty FeMoco (gray). (Right) Experimental XES data (gray) provide best match with predicted carbon spectrum (black).[11]

The recent assignment of the central atom of FeMoco is an extremely important discovery in our understanding of nitrogenase. With the structure of the active site of nitrogenase elucidated, the incorporation of iron-carbon interactions into functional model complexes will help to elucidate the functional consequences of the interstitial carbon atom on nitrogenase activity. This will hopefully lead to the development of better catalysts to enable more efficient nitrogen reduction under more moderate conditions.

(1) Crossland, J. L.; Tyler, D. R. Iron-dinitrogen coordination chemistry: Dinitrogen activation and reactivity, *Coord. Chem. Rev.* **2010**, *254*, 1883-1894.
(2) Ramaswamy, S. One Atom Makes All the Difference, *Science* **2011**, *334*, 914-915.
(3) Kim, J.; Rees, D. Structural models for the metal centers in the nitrogenase molybdenum-iron protein, *Science* **1992**, *257*, 1677-1682.
(4) Seefeldt, L. C.; Hoffman, B. M.; Dean, D. R. Mechanism of Mo-Dependent Nitrogenase, *Annu. Rev. Biochem.* **2009**, *78*, 701-722.
(5) Einsle, O.; Tezcan, F. A.; Andrade, S. L. A.; Schmid, B.; Yoshida, M.; Howard, J. B.; Rees, D. C. Nitrogenase MoFe-Protein at 1.16A Resolution: A Central Ligand in the FeMo-Cofactor, *Science* **2002**, *297*, 1696-1700.
(6) Lee, H.-I.; Benton, P. M. C.; Laryukhin, M.; Igarashi, R. Y.; Dean, D. R.; Seefeldt, L. C.; Hoffman, B. M. The Interstitial Atom of the Nitrogenase FeMo-Cofactor: ENDOR and ESEEM Show It Is Not an Exchangeable Nitrogen, *J. Am. Chem. Soc.* **2003**, *125*, 5604-5605.

(7) Yang, T.-C.; Maeser, N. K.; Laryukhin, M.; Lee, H.-I.; Dean, D. R.; Seefeldt, L. C.; Hoffman, B. M. The Interstitial Atom of the Nitrogenase FeMo-Cofactor: ENDOR and ESEEM Evidence That it is Not a Nitrogen, *J. Am. Chem. Soc.* **2005**, *127*, 12804-12805.
(8) Lukoyanov, D.; Pelmenschikov, V.; Maeser, N.; Laryukhin, M.; Yang, T. C.; Noodleman, L.; Dean, D. R.; Case, D. A.; Seefeldt, L. C.; Hoffman, B. M. Testing if the Interstitial Atom, X, of the Nitrogenase Molybdenum-Iron Cofactor Is N or C: ENDOR, ESEEM, and DFT Studies of the S = 3/2 Resting State in Multiple Environments, *Inorg. Chem.* **2007**, *46*, 11437-11449.
(9) Dance, I. Electronic Dimensions of FeMo-co, the Active Site of Nitrogenase, and Its Catalytic Intermediates, *Inorg. Chem.* **2010**, *50*, 178-192.
(10) Delgado-Jaime, M. U.; Dible, B. R.; Chiang, K. P.; Brennessel, W. W.; Bergmann, U.; Holland, P. L.; DeBeer, S. Identification of a Single Light Atom within a Multinuclear Metal Cluster Using Valence-to-Core X-ray Emission Spectroscopy, *Inorg. Chem.* **2011**, *50*, 10709-10717.
(11) Lancaster, K. M.; Roemelt, M.; Ettenhuber, P.; Hu, Y.; Ribbe, M. W.; Neese, F.; Bergmann, U.; DeBeer, S. X-ray Emission Spectroscopy Evidences a Central Carbon in the Nitrogenase Iron-Molybdenum Cofactor, *Science* **2011**, *334*, 974-977.
(12) Spatzal, T.; Aksoyoglu, M.; Zhang, L.; Andrade, S. L. A.; Schleicher, E.; Weber, S.; Rees, D. C.; Einsle, O. Evidence for Interstitial Carbon in Nitrogenase FeMo Cofactor, *Science* **2011**, *334*, 940.