

If the Cluster Were a Cluster: The Active Centre of Nitrogenase Revisited

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The structure and function of the FeMo cofactor of nitrogenase continue to be a challenge in bioinorganic chemistry.^[1] Here the electronic and physical structure of the active centre are described from an inorganic point of view, the central main-group element is confirmed and its role within the cluster is explained.

The recent structure determination of the nitrogenase FeMo cofactor by Schmid, Rees and co-workers at an atomic resolution of 116 pm found a second-row element "E" in the centre of the trigonal prismatic arrangement of the six iron atoms that form the core of the active centre of the FeMo cofactor.^[2] The authors tentatively assigned this element as nitrogen, since their theoretical calculations gave nitrogen as the closest fit, but with carbon and even oxygen as very close alternatives. Let us reconsider the structure of the cluster from a purely inorganic point of view. "A metal atom cluster may be defined as a group of two or more metal atoms in which there are covalent bonds between the metal atoms."^[3]

The overall structure of the FeMo cluster (see Figure 1) is a trigonal prismatic arrangement of six iron atoms.^[2] The three vertical edges of the square-planar faces of the prism are bridged by sulfur atoms. The two trigonal faces are capped by an iron and a molybdenum atom to create two metal tetrahedra (Fe₄ and Fe₃Mo). The six outward-facing trigonal faces of these two metal tetrahedra are capped by sulfur atoms. The central main-group, second-row element in the centre of the trigonal prism com-

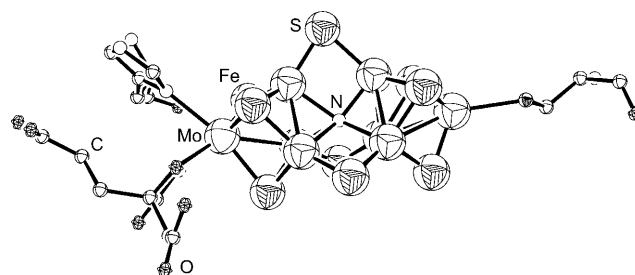


Figure 1. The active centre of the FeMo cofactor of nitrogenase. Atoms are shown with arbitrary size, hydrogen atoms have been removed for clarity. The cluster is connected to the protein by Cys275 (right) and His442 (left) and bears a homocitrate ligand on molybdenum.

pletes the structure of the cluster. The homocitrate, a histidine and a cysteine residue on the apical molybdenum and iron atoms complete the electron count. The cluster is bound to the protein through the histidine and cysteine residues.

A "trigonal prismatic" arrangement of six iron atoms^[4] can be found as part of cubic space-centred packing (W-type) in which α -iron crystallises.^[5] The second-row element occupies a trigonal-prismatic hole within the lattice. Other trigonal-prismatic holes (on the surface of the trigonal prism) are occupied by the apical metal atoms (Fe and Mo). The three bridging sulfur atoms occupy octahedral interstices in the α -iron lattice. The six sulfur atoms capping the two tetrahedra occupy positions that are not found in α -iron.

As to the nature of the second-row element in the centre of the trigonal prism, we can again turn to our knowledge of inorganic chemistry.^[5] The solubility of carbon in α -iron is only 0.018% at 738 °C; this means that carbon does not tolerate trigonal prismatic holes.^[6] Instead, it occupies octahedral holes in intercalation carbides with γ -iron. Similarly, oxygen is not found in trigonal prismatic interstices.

The structure of the FeMo cofactor of nitrogenase is essentially based on the structure of α -iron, and we would expect that the bond lengths would be in line with Fe/Mo–Fe, Fe/Mo–S and Fe–E (E = N, C, O) covalent bonds. The covalent radii of Fe, Mo, S and N are 124, 136, 104 and 70 pm, respectively.^[5] This results in calculated bond lengths of 248 pm (Fe–Fe), 260 pm (Fe–Mo), 228 pm (Fe–S), 240 pm (Mo–S) and 194 pm (Fe–N). The bond lengths for Fe–O and Fe–C are 190 and 201 pm, respectively. Schmid, Rees and co-workers found a Fe–E distance of 200 pm and a S–E distance of 330 pm, which leads to an Fe–S bond length of 235 pm.^[2] The Fe/Mo–Fe bond lengths were found to be 258–273 pm, with the capped M–M bonds shorter than the bridged ones, as expected. All experimentally determined bond lengths are approximately equivalent to the sum of the covalent radii, and we can assume covalent bonds between nearest neighbours.

Having established the geometrical features of the cluster in the FeMo cofactor, we need to determine the electronic structure, with the assumption that the cluster unit is uncharged. This assumption is based on the fact that no counterion was found in the crystal structure; to assume a remote counter charge

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somewhere on the peptide chain is not appropriate. First, we have to remember that the archetypical cluster is the borane cluster in which the BH unit possesses four electrons. Two electrons are located in the B–H bond pointing away from the cluster and two electrons are contributed towards the cluster skeleton (the cluster framework). From the isolobal relationship, it follows that a BH unit with four electrons is equivalent to a transition metal fragment with 14 VE.^[7] A sulfur atom has a lone pair that points away from the cluster, leaving four electrons that can be used in bonding to the metal atoms. With the further assumption that the cluster in the FeMo cofactor is a *conjuncto* cluster obeying Wade's rules,^[7] we can count the inorganic sulfur atoms as four-electron donors, the cysteine sulfur atom as a one-electron donor, the histidine nitrogen atom as a two-electron donor and the homocitrate ligand as contributing two electrons towards the electron count of the transition metals.

We can now turn to the cluster itself, which can be divided into three sections for closer inspection (see Figure 2), the two tetrahedra and the central trigonal prism. The apical Fe atom has, as a group-eight metal, eight valence electrons. A ninth electron is contributed by the cysteine residue, and the three capping sulfur atoms need to contribute a further five electrons to arrive at the 14

electrons required for a transition metal in a *closo* cluster. The three Fe atoms at the base of the capping tetrahedron also have eight electrons each and receive a total of seven electrons from the three capping sulfur atoms. That is 2.33 electrons each, making the total 10.33 electrons per iron atom. As these iron atoms are also part of the central trigonal prism, they receive their share of the twelve electrons available from the three sulfur atoms bridging this central unit. These twelve electrons are equally divided amongst all six iron atoms, giving each iron atom two electrons. This makes a total of 12.33 electrons for each of the three iron atoms of the Fe₄ tetrahedron. That is 0.67 electrons short of the 13 electrons required for a transition metal in the bridgehead position of a *conjuncto* cluster.

We now turn to the bottom half of the cluster in the FeMo cofactor. The Mo atom of the Fe₃Mo tetrahedron has six electrons of its own and receives a total of four electrons from the histidine and the homocitrate, making the total count ten electrons. The missing four electrons are contributed by the three capping sulfur atoms; this leaves them with eight electrons. These electrons are equally divided amongst the remaining three Fe atoms to give them 10.67 electrons each. Two electrons each from the three sulfur atoms bridging the trigonal prism bring the total count up to 12.67 elec-

trons, 0.33 electrons short of the 13 electrons required for a transition metal in the bridgehead position of a *conjuncto* cluster.

We note that our electron count reveals a shortage of three electrons for the central trigonal prism unit. The role of the "mysterious" main-group element E in the centre of the cluster becomes obvious now. It is there to provide the three missing electrons.

Before we decide on the nature of E, we will recount our cluster on the basis of ionic charges. We first calculate the total anionic charge. The inorganic sulfur atoms are S²⁻ with two negative charges, the cysteine unit carries one negative charge and the homocitrate has two negative charges. That makes a total of 21 negative charges, excluding the central atom E. This total negative charge has to be balanced by the oxidation states of the eight metal atoms, and the negative charge of E must be taken into account—the latter an uncertainty at the moment. The oxidation state of molybdenum has been determined as +IV;^[2] thus leaving 17 positive charges to be divided amongst the seven iron atoms. We arrive at four Fe^{II} and three Fe^{III} atoms. Such an arrangement would create a completely asymmetric Fe₆ trigonal prism at the centre of the cluster, a clear violation of experimental findings, which call for near perfect symmetry. This situation is remedied by assigning one Fe^{II} and six Fe^{III} atoms to create three additional positive charges. This makes the central main group atom an E³⁻ entity, most likely a nitride, if our assumption of a neutral cluster unit is correct. A boride would of course principally be possible, although possibly too small to fit comfortably into the hole provided, whereas the higher homologues of nitrogen are certainly too big.

For nitrogen to contribute three electrons to the cluster, we would need to assume that the nitrogen atom was not hybridised, but contributed the electrons via the three p orbitals, retaining a lone pair with s character. This situation is common with the higher homologues (As, Sb and Bi, and, to a lesser extent, P) and in keeping with the main-group chemistry of elements in the fourth, fifth and sixth periods, but unusual for nitro-

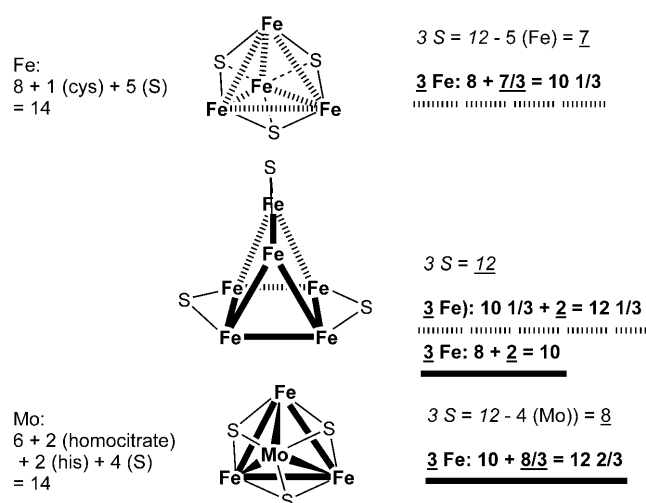


Figure 2. The electron count within the Fe₇MoNS₉ cluster of the FeMo cofactor. The bond style represents the three sections of the cluster: Fe₄, Fe₆ and Fe₃Mo. Each iron atom possesses eight electrons, and each sulfur atom contributes four electrons towards bonding with the metal atoms.

gen. However, the alternative, a sp^3 lone pair with deformation of the cluster, seems even more unlikely.

We conclude that the FeMo cofactor of nitrogenase can be described as a *closo-conjuncto* cluster of the composition Fe_7MoNS_9 attached to the protein via a histidine (Mo) and a cysteine (Fe) amino acid. In this model, electronic saturation, on molybdenum, is achieved by the homocitrate. The iron atoms are eight-coordinate, except for the apical iron atom, which is seven-coordinate. The molybdenum atom is nine-coordinate. The central nitride ion provides three electrons to the *closo-conjuncto* cluster, and it is very unlikely that it plays any role in the reduction of atmospheric nitrogen.

A *closo-conjuncto* cluster provides energetically closely spaced molecular orbitals capable of mediating multistep, multielectron redox reactions, as is required

in the reduction of atmospheric nitrogen to ammonia.

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Keywords: enzymes · iron · metal clusters · molybdenum · nitrogenase

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- [3] F. A. Cotton, G. Wilkinson, *Advanced Inorganic Chemistry*, 5th ed., Wiley, Chichester, **1988**.
- [4] Of course, the “trigonal prismatic” arrangement in α -iron is not a true, regular trigonal prism, but it can be described as a trigonal prism in which one vertical edge is pushed upward so that the trigonal base forms a 45° angle (instead of 90°) with the square-planar (unmoved) face. Obviously, outside the con-

straints of the metal packing, this arrangement is free to relax into the regular trigonal prism.

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- [6] The *Encyclopedia of Inorganic Chemistry* refers to the structure of cementite (Fe_3C) in the following way: “In the irregular structure of cementite, carbon is located in a very distorted trigonal prism...” However, the crystal structure of cementite (<http://www.msm.cam.ac.uk/phase-trans/2003/Lattices/cementite.html>) is very regular and contains polyhedra that can be described as bicapped trigonal prisms. In these polyhedra, carbon has eight iron atoms in the first coordination sphere and not six, as in a trigonal prism; this means that the description of the structure as a very distorted trigonal prism is misleading. *The Encyclopedia of Inorganic Chemistry*, 2nd ed., (Ed.: R. B. King) Wiley, New York, **2005**.
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