A significant breakthrough has occurred in the crystallographic analysis of the iron-molybdenum protein of nitrogenase. The overall distribution of the metal clusters in the protein is shown in Figure 7.40. The distance between the two FeMoco units is fully consistent with each cofactor acting as an independent active site. On the other hand, the closeness of the P cluster and FeMoco centers in each unit is indicative of their likely cooperation in the N₂ fixation reaction.

Figure 7.40 - A schematic representation of the spatial arrangement of the metal sulfur clusters bound to Cpl as determined by the x-ray anomalous scattering studies described in the text. The representation of the large, "8-Fe" cluster with a P symbol indicates only that it must contain the Fe atoms normally assigned to P-clusters.

The proposed structure of the P cluster, shown in Figure 7.41, involves a doubly bridged, double cubane unit consisting of one normally bound Fe₄S₄ cluster with all cysteine ligands and one Fe₄S₄ cluster that contains an unusual cysteine/serine (S/O) ligand pair on one of its two nonbridged Fe positions. Such five-coordinate iron in an Fe₄S₄ cluster is not unprecedented. The two Fe₄S₄ clusters are disposed to produce a face-sharing arrangement with two cysteine ligands bridging the two sets of Fe atoms. An interesting feature of the structure is a disulfide unit linking the two clusters; this unit potentially could be redox-active during nitrogenase turnover.

Figure 7.41 - Proposed P-cluster pair in A. vinelandii FeMo protein.

Most striking of the new results is the proposed structure of FeMoco shown in Figure 7.42. The cluster core of composition Fe₇MoS₈ can be viewed as two halves bridged by two S²⁻ ions and an unknown ligand (designated Y in the figure). The MoFe₃S₃ half of the core is in the shape of a thiocubane fragment missing one \(\mu_3\)-S²⁻ ion. The Mo is six coordinate; the ligands are three \(\mu_2\)-S²⁻ ions, which bridge to the three Fe ions, an \(\alpha\)-His-442 nitrogen,
and two oxygen donors (the hydroxyl and central carboxylate) of the homocitrate ligand. Interestingly, the second half of FeMoco is a similar thiocubane fragment, Fe₄S₃, also missing a \(\mu_3\)-S²⁻ ion. This unit has a single noncore ligand, \(\alpha\)-Cys-275, which is bound to the terminal Fe atom of the cluster. The two thiocubane fragments (MoFe₃S₃ and Fe₄S₃) are bridged by three ligands in a face-sharing mode with the two Fe₃ faces eclipsed with respect to each other. The eight metal ions display a bis(end-capped) trigonal prismatic arrangement with three bridges on the edges of the prism, which connect the two thiocubane fragments. The two sulfide bridges between the thiocubane halves are clearly defined in the structure, but the third bridge is not, suggesting the possibility that this is in fact part of the N₂-binding site. Interestingly, \(\alpha\)-His-195, identified as essential for N₂ fixation by mutagenesis and ESEEM studies, does not appear to be covalently bound, although it is close to the FeMoco unit.

Figure 7.42 - Proposed cofactor cluster in A. vinelandii FeMo protein.

Clearly, this structure is not the same as any of those previously proposed (Figure 7.31), although it does possess many features that were identified in model studies. While it is tempting to speculate that the central bridge of the cluster (the Y ligand) is the site of N₂ reduction, this is in no way established at present. The structural definition of the nitrogenase proteins is now progressing at a rapid rate. Many of the physical measurements will have to be reexamined in light of the new data. Through further experimentation involving physical methods, mutagenesis, and kinetic/mechanistic studies, much more information about the role of ATP, the activation of hydrogen, and the binding, activation, and reduction of N₂ and other nitrogenase substrates should be obtained.