Supporting Material

Materials and methods

MoFe protein from aerobically grown cells of *Azotobacter vinelandii* was purified as described previously (*1*). Crystals were obtained by equilibrating a reservoir solution containing 13% polyethylene glycol 8000, 1 M sodium chloride and 0.1 M Tris-hydroxyethyl-aminomethane/HCl buffer at pH 8.0 against 8 µl of a 1:1 mixture of 30 mg/ml of MoFe protein and the reservoir solution under strictly anaerobic conditions. For flash-cooling, the crystals were successively transferred into harvesting solutions consisting of the reservoir solution and increasing amounts of 2-methyl-2,4-pentanediol (MPD). This was carried out in steps of 5 % MPD for 15 min each, until 20 % MPD were reached. Crystals were reduced by addition of 10 mM sodium dithionite to the cryo buffers.

Data were collected on beamline 9-2 at the Stanford Synchrotron Radiation Laboratory, with a Quantum-315 charge-coupled device detector at a wavelength of 0.998 Å. Integration and scaling were carried out with the HKL suite (*2*), showing that the voume of the unit cell was doubled in comparison to the previously determined structures from the same organism. Phase information was obtained by molecular replacement with the program MOLREP (*3*), using *A. vinelandii* MoFe protein at 2.0 Å (PDB-code 3MIN) as a search model. Initial refinement was carried out with CNS (*4*), and in later stages alternative conformations and anisotropic B-factors were refined with REFMAC (*5*). Model building was done using O (*6*).

Theoretical resolution-dependent electron density profiles were calculated using Waterloo Maple 7, while profiles derived from the refined structures were obtained using programs from the CCP4 suite (*7*).

References

1. D. Wolle, C. Kim, D. Dean, J.B. Howard, *J. Biol. Chem.* **267**, 3667-3673 (1992).
2. Z. Otwinowski, W. Minor, *Methods Enzymol.* **276**, 307-326 (1996).
3. A. A. Vagin, A. Teplyakov, *J. Appl. Crystallogr.* **30**, 1022-1025 (1997).
4. A. T. Brünger *et al.*, *Acta Cryst.* **D54**, 905-921 (1998).
5. G. N. Murshudov, A. A. Vagin, E. J. Dodson, *Acta Cryst.* **D53**, 240-255 (1997).
6. T. A. Jones, J.-Y. Zou, S. W. Cowan, M. Kjelgaard, *Acta Cryst.* **A47**, 110-119 (1991).
7. Collaborative Computational Project No. 4, *Acta Cryst.* **D50**, 760-763 (1994).