Breaking symmetry: A path towards understanding cooperativity in nitrogenase.

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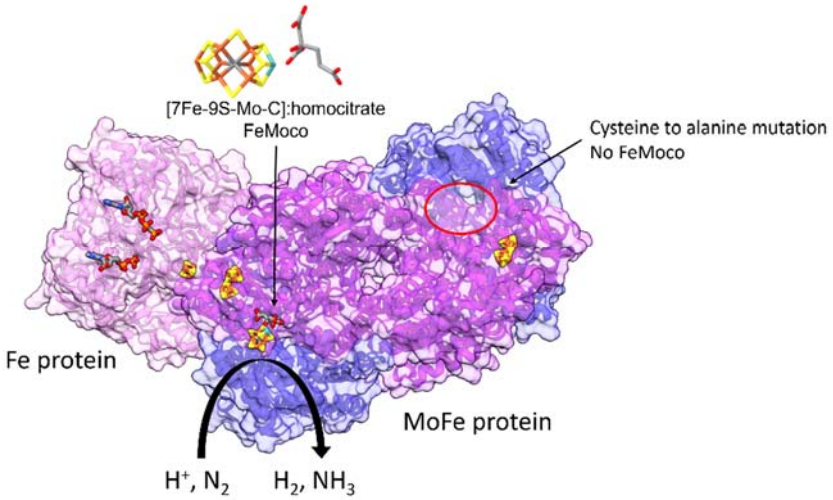
Nitrogenases are the only enzymes capable of reducing inert dinitrogen (N_2) to ammonia (NH_3). Such a feat can only be achieved via a complex mechanism which is still under debate. The enzymatic complex of nitrogenases consists of two separated proteins: a reductase, iron protein (or Fe protein) and a catalytic component, molybdenum-iron protein (or MoFe protein).

The focus of this study is the MoFe protein and its heterotetrameric and symmetric $\alpha_2\beta_2$ arrangement. Each αβ half of the protein is catalytically active[1] and contains in its α-subunit, an unique metallocofactor [7Fe-9S-Mo-C]:homocitrate labeled FeMoco were substrate reduction occurs. The removal of this cofactor by single mutation of an apical coordinating cysteine to an alanine completely suppresses activity.[2] Removing the cofactor on one side only would allow to understand if activity on one side is affected by being paired with an inactive half, probing the presence and the nature of cooperativity in nitrogenase MoFe protein.[3]

Two different approaches towards producing asymmetric MoFe protein lacking cofactor on one half were developed and assayed. The results showed that half-active asymmetric MoFe protein exhibits > 50% activity when compared to complete protein, bringing good evidence for negative cooperativity between the two halves of the MoFe protein.



References:

[1] O. Einsle, D. C. Rees, *Chem. Rev.* **2020**, *120*, 4969–5004
[2] H. M. Kent, M. Baines, C. Gormal, B.E. Smith, M. Buck, *Mol. Microbiol.*. 199**0**, *4,* 9,1497-1504.
[3] C. Cadoux, D. Ratcliff, N. Maslać, W. Gu, I. Tsakoumagkos, S. Hoogendoorn, T. Wagner, R. D. Milton *JACS Au.* **2023**, *3*, 5, 1521-1533.