# Nitrogenase biosynthesis: keeping two [4Fe:4S] clusters close before their fusion into the P-cluster

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Dinitrogen binding and reduction at the nitrogenase active site require successive electron transfer steps via the [7S:8Fe] P-cluster, located at the $\alpha/\beta$ interfaces of the enzymatic complex. Although the P-cluster displays structural similarities to the cofactors of nitrogenases, they have a distinct biosynthetic pathway, which is still not fully understood. Contrary to the cofactors, which are synthesized and fully maturated on distinct scaffolds before their transfer to the enzyme, P-clusters are maturated *in situ* from two [4S:4Fe] cubanes. Furthermore, studies on molybdenum nitrogenase have suggested that P-cluster maturation is a prerequisite for FeMo-co insertion in the catalytic moiety ($NifD_2K_2$).[1] In the soil bacterium *Azotobacter vinelandii*, three accessory proteins sequentially bind NifDK before the final P-cluster maturation step catalysed by the iron protein $NifH_2$: NafH, NifW, and NifZ.[2] The function of these proteins remains unclear.

To shed light on the role of the NifW accessory protein, we built an *A. vinelandii nifH*/*nifZ* deletion strain and isolated $NifD_2K_2$ from this strain in several fractions: bound to NifW, to NafH, or to no accessory factor. We characterized those immature $NifD_2K_2$ species via EPR and X-ray absorption spectroscopy. In both unbound and NifW-bound NifDK, we identified the cluster species as two distinct [4S:4Fe] clusters, whose coupling increases upon NifW binding. Our data lead us to propose that NifW plays a structural role in P-cluster maturation, bringing two cubane precursors closer upon a conformation change of NifDK. These results further clarify the biosynthesis of one

[1] Markus Ribbe, Yilin Hu, Maolin Guo, Benedikt Schmid, Barbara Burgess, *J. Biol. Chem.*, **2002**, *277(26)*, 23469-23476.

[2] Emilio Jimenez-Vicente, Zhi-Yong Yang, W. Keith Ray, Carlos Echavarri-Erasun, Valerie L. Cash, Luis M. Rubio, Lance C. Seefeldt, Dennis R. Dean *J. Biol. Chem.*, **2018**, *293(25),* 9812–9823.

of Nature's most complex metalloclusters, and may be essential for heterologous production of nitrogenase.[3]

[3] Stefan Buren, Eric M. Young, Elizabeth A. Sweeny, Gema Lopez-Torrejon, Marcel Veldhuizen, Christopher A. Voigt, Luis M. Rubio, *ACS Synth. Biol.*, **2017**, *6*, 1043−1055.