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Nitrous oxide reductase with a unique [4Cu:2S] centre from the denitrifying Pseudomonas stutzeri

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Denitrification is the microbiological reduction of nitrate via nitrite, nitric oxide and nitrous oxide, followed by the subsequent two-electron reduction of nitrous oxide to dinitrogen.
Nitrous oxide reductase, NosZ, is a dimeric multi-copper protein with 638 residues per subunit and the reported Cu content depends on the purification strategy. Because of the sensitivity of the enzyme toward dioxygen, the clusters of the soluble periplasmic enzyme degrade and it therefore loses its activity under oxic conditions. Several different forms were described, that can be distinguished by their typical absorption and EPR spectra. The active purple form of the enzyme carries the well-characterized mixed-valent binuclear CuA centre and the tetranuclear CuZ site, that was first described as a unique [4Cu:2S] centre for Pseudomonas stutzeri, instead of a [4Cu:S] cluster5 found previously. This cluster was observed after the isolation and crystallization under the exclusion of dioxygen. In nitrous oxide reductase the substrate N2O is bound between the two copper centres, it is activated by side-on binding to CuZ, so that then electrons can be transferred directly from CuA to the N2O. To determine the unknown mechanistic details, several spectroscopic methods can be used to take a closer look at different redox states of the copper ions. Several accessory proteins were identified for the biogenesis of the active N2O reductase, with predicted functions as Cu chaperone or ABC transporters.

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