

## NMR Solution Structure of the *Desulfovibrio gigas* Orange protein

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The Orange protein from *Desulfovibrio gigas* was isolated from a large scale growth cultured in a lactate-sulphate medium under anaerobic conditions. It is an orange coloured 11.8 kDa protein (117 residues) that contains a mixed-metal sulphide cluster, of the type  $[S_2MoS_2CuS_2MoS_2]^{3-}$ , non-covalently bound to the polypeptide chain<sup>1,2</sup>.

A blast search revealed that this protein has sequence homology of around 30 to 40 % with conserved proteins from eubacteria and hyperthermophilic archae with unknown function. They all contain a conserved domain common to the nitrogenase accessory factor (NifB C-terminal domain, NifX and NafY).

The Orange protein was produced for NMR studies by heterologously expression in *E. coli* as the apo-form<sup>3</sup>. The holo form was reconstituted *in vitro* by the addition of copper and thiomolybdate.

The polypeptide chain of Orange protein is composed of 121 residues, 117 residues plus 4 that were added by the cloning procedure, while, 5 are prolines. From the expected 114 amide proton resonances, only 106 or 108 were observed in the  $^1H$ - $^{15}N$  HSQC of the apo-protein and reconstituted protein, respectively. This is probably due to exchange with the bulk solvent. The residues with non-observable amide resonances were assigned with the aid of  $^{13}C$  detection experiments, which also enabled the extension and confirmation of the sequential assignment for both forms of the protein<sup>3</sup>. The over-all solution structures of the apo and reconstituted Orange protein are similar, with an  $\alpha/\beta$  motif, characteristic of the members of the ribonuclease H family. Although, the two structures are similar, mapping of the chemical shift differences between them provides a way to elucidate which region of the polypeptide chain is involved in the binding of the metal cluster.

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