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HYBRID CLUSTER PROTEIN (HCP) FROM *DESULFOVIBRIO VULGARIS* AND *DESULFOVIBRIO DESULFURICANS*: AEROBIC, ANAEROBIC AND REDUCED STRUCTURAL STUDIES

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A novel Fe-S cluster was first identified in protein referred to as Hybrid Cluster Protein (HCP) purified from the strictly anaerobic sulphate reducing bacterium Desulfovibrio vulgaris1,2. The crystal structure to 1.6 resolution showed the protein to contain two Fe-S clusters, one conventional 4Fe-4S cubane cluster and the novel cluster, a hybrid combining iron-sulphide and iron-oxo substructures3. The anomalous magnetic properties and the very high-spin EPR signal of this protein made it of special and unique interest. Both the reduced and as-isolated anaerobic Desulfovibrio desulfuricans HCP structures have been solved to 1.25 resolution5, and confirm the presence of both the cubane and the hybrid clusters found in the D. vulgaris protein. Desulfovibrio vulgaris HCP structures reduced and anaerobic isolated were also solved recently up to 1.35 **Å** resolution. Sequence alignments with CO-dehydrogenases revealed the conservation of almost all Fe-S cluster binding residues, raising the possibility of their functions being related.

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ATOMIC RESOLUTION STRUCTURE OF A RUSTICYANIN MUTANT FROM *THIOBACILLUS FERROXIDANS*

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Rusticyanin is a type I blue copper protein and is thought to be a principal component in the iron respiratory chain of *Thiobacillus ferroxidans*. It contains a single polypeptide chain with a copper atom as the prosthetic group and is composed of a core beta-sandwich along with a 35 residue N-terminal extension. Rusticyanin has exceptional acid stability and its optimal pH is between 1 and 3. In addition to this remarkable property, it has a Cu(I)/(II) redox couple of around 680mV whereas more typical redox potentials for type I copper proteins are around 300mV. The copper coordination is that of a distorted trigonal planar geometry with three strong ligands, His85Nd1, Cys138Sg and His143Nd1 and a relatively weaker, axial Met148Sd. The His143Met mutation has led to crystals which diffract to 1.1 Angstrom, significantly improving the resolution over the structure of native Rusticyanin. In fact, this represents the highest resolution structure of a cupredoxin to date. Detailed analysis of this atomic resolution structure would be presented.
The mutant has also been crystallized in a different form with two molecules in the crystallographic asymmetric unit. Even though, this crystal form diffracts to a lower resolution (2.3 Å), its structure determination has provided important information about a possible electron transfer route with a redox partner.

Keywords: **RUSTICYANIN, ATOMIC RESOLUTION, CUPREDOXIN**

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CRYSTAL STRUCTURE FROM THE MOLYBDENUM-COPPER COFACTOR OF THE ORANGE PROTEIN

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The 'orange' protein from *Desulfovibrio gigas* is an 11.9 kDa protein of unknown function, which can be isolated from the organism grown on lactate-sulfate medium under anaerobic conditions. The protein contains a novel molybdenum-copper cofactor with unknown structure. A structure for this cofactor has been proposed based on EXAFS data on the protein-cofactor complex (George et al., 2000), which comprises a $[S_2MoS_2CuS_2MoS_2]^{3-}$ cluster.
While attempting the crystallization of the protein, small ruby crystals appeared, with dimensions 0.02x0.02x0.0005 mm. Analysis of the crystals showed that they did not contain protein but the extruded cofactor. X-ray diffraction experiments were performed on the ID14-4 beamline at the ESRF synchrotron facility in Grenoble. The crystal structure was solved by direct methods and was found to contain a cubic [8Mo-12Cu-32S] cluster and organic counter-ions.
Apparently, the cofactor-protein structure was disrupted after which the cofactor crystallized, whereas the protein precipitated. The structure solved by us is directly related to the cofactor of the orange protein and is perhaps the cofactor itself. The structures postulated by George et al. (2000) and by us will be presented on the poster.
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PH DEPENDENCE OF PEROXIDE DERIVED HIGH VALENT HAEM IN MYOGLOBIN STUDIED BY X-RAY CRYSTALLOGRAPHY

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The biological conversions of O_2 and peroxides to water as well as certain incorporations of oxygen atoms into small organic molecules can be catalyzed by metal-ions in different clusters or cofactors. The catalytic cycles of these reactions pass through similar metal-based complexes in which one oxygen- or peroxide-derived oxygen atom is coordinated to an oxidized form of the catalytic metal-center. In haem-based peroxidases or oxygenases the ferryl ($Fe^{IV}O$) form is important in the compound I and compound II complexes, which are two and one oxidation equivalents higher than the ferric (Fe^{III}) form, respectively. In this study we report high resolution X-ray structures (to 1.35 Å) of a compound II model protein, obtained by reacting ferric myoglobin with hydrogen peroxide at pH 5.2, 6.8 and 8.7 [1]. The molecular geometry is virtually unchanged compared to the ferric form, indicating that these reactive intermediates do not undergo large structural changes. The essential Fe---O distance is 1.9 Å at all pH-values, which is long compared to the 1.6 Å distance in the oxy-ferryl (Fe^{IV}=O) species commonly used as a model for compound II. Based on these observations we propose that compound II is a hydroxyl-ferryl iron (Fe^{IV}-OH). The 1.9 Å Fe---O distance is in agreement with an EXAFS study of compound II in horseradish peroxidase [2].
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