s8a.m7.o1 **Recent Advances in Metalloprotein Crystallography.** A. Messerschmidt, *Max-Planck-Institut für Biochemie, D-82152 Martinsried*
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Metalloprotein crystallography has tremendously benefited from recent advances in synchrotron radiation techniques for macromolecular crystallography. The state of the art of these techniques allows the routine application of the multiwavelength anomalous diffrac-tion (MAD) method. In the case of metallo-proteins, which normally contain one or several intrinsic anomalous scatterers, one crystal of the native protein is sufficient in principle to determine the complete x-ray structure.

The application of the MAD technique will be presented by the structure determination of the dissimilatory cytochrome *c* nitrite reductase, a pentaheme enzyme from *Sulfurospirillum deleyianum.* This enzyme cataly-zes the six-electron reduction of nitrite to ammonia as one of the key steps within the biological nitrogen cycle. This structure was solved at 1.9 Å resolution[1]. The protein is a homodimer with 10 hemes in very close packing, with the nearest Fe-Fe distance of 9 Å. All hemes are bis-histidinyl-coordinated except the catalytic heme which binds a lysine in 5th place as a novel structural motif, and a sulfate anion at the subtrate binding site. The extraordinarily high activity of the enzyme can be rationalized by the presence of a channel that separates access for substrate anions and product cations by its electrostatic surface potential.

The MAD technique has been successfully applied to identify the nature of bound metals in metalloproteins. An excellent example is the structure analysis of Ni-Fe hydrogenase, where the second ion in the binuclear metal center could be assigned to be iron[2].

A further important issue is the preparation and structural analysis of functional derivatives of metalloproteins. This will be illustrated by the structure determination of the peroxide derivative of chloro-peroxidase from the fungus *Curvularia inaequalis*[3]. This enzyme belongs to the group of haloperoxidases, which oxidize halides in the presence of hydrogen peroxide to the corresponding hypohalous acids. In the 2.24 Å crystal structure the peroxide is bound to the vanadium in an η^2-fashion after the release of the apical oxygen ligand. The coordination geometry around the vanadium is that of a distorted tetragonal pyramid with the two peroxide oxygens, one oxygen and a nitrogen in the basal plane and one oxygen in the apical position. The X-ray structures of the native and peroxide forms build the structural basis for the understanding of the catalytic mechanism.

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s8a.m7.o2 **A Novel Type of Catalytic Copper Cluster in Nitrous Oxide Reductase.** K. Brown[1], M. Tegoni[1], M. Prudêncio[2], A. Pereira[2], S. Besson[2], J. Moura[2], I. Moura[2] and C. Cambillau[1]. [1]*Architecture et Fonction des Macromolécules Biologiques (AFMB), UPR 9039 CNRS, Marseille, France* [2]*Departamento de Quimica, Universidade Nova de Lisboa, Portugal*
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In the absence of oxygen, denitrifying bacteria, like *Pseudomonas* and *Paracoccus*, are able to obtain energy by coupling the reduction of nitrogen oxide species (NO_3^-, NO_2^-, NO, N_2O) to the formation of ATP. The denitrification process is crucial for the conversion of nitrates, used as fertilizers and pollutants of ground and surface water, to gaseous species like NO or N_2O and for the return of large amounts of N_2 to the atmosphere.

Nitrous oxide reductase, catalyses the last step in anaerobic denitrification: the reduction of the greenhouse gas N_2O to N_2. The enzyme from *Pseudomonas nautica* is a homodimer (2x65kDa) and contains a di-nuclear CuA centre, homologous to that of subunit II of cytochrome oxidase, which is the electron acceptor pole and a catalytic CuZ centre, supposed di-nuclear and of unknown structure. We have solved the structure to 2.4Å resolution using the copper anomalous signal and the technique of multiple wavelength anomalous dispersion. Each monomer if nitrous oxide reductase is composed from two distinct domains formed from contiguous segments in the amino-acid sequence (Fig 1.); the N-terminal domain, a 7-bladed β-propellor, and the C-terminal domain with a cupredoxin fold. The structure reveals that the CuZ centre is located on top of the β-propeller domain and has a totally new structure: four-copper ions arranged in a distorted tetrahedron are co-ordinated by seven histidine residues and three OH^- ions (Figure 1). The CuA centre is confirmed as a di-nuclear bis-thiolate bridged copper cluster, and is situated in a loop region between strands in the C-terminal domain. Nitrous oxide reductase is a dimer, and as a result of intermonomer 'domain exchange', the CuA centre of one monomer is in close proximity to the CuZ centre of the second monomer (closest distance 10.2Å)

On the basis of the structure, we can propose a mechanism in which N_2O binds by its oxygen extremity to one of the copper atoms of the CuZ cluster, the remaining Cu ions functioning as an electron buffer, favouring rapid electron exchange and preventing the formation of dead-end products.

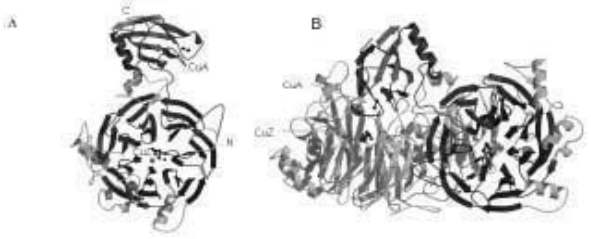

Fig 1 : Structure of the reduced nitrous oxide reductase from *P. nautica.* (A) Overall view of the monomer, showing the CuA and CuZ copper clusters. (B) View of the homodimer; one monomer is uniformly coloured grey, while the other has the same colour codes as in A.
Kieron Brown, Mariella Tegoni, Miguel Prudêncio, Alice Pereira, Stephane Besson, José Moura, Isabel Moura and C. Cambillau (2000) *Nature Structural Biology*, **7 (3)**, 191-195