Crystal structure of 6-hydroxy-L-nicotine oxidase from *A. nicotinovorans* at 2.0 Å resolution

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Arthrobacter nicotinovorans is able to use nicotine as its only source of carbon and oxygen. Two flavoproteins, 6-hydroxy-L-nicotine oxidase and 6-hydroxy-D-nicotine oxidase, which are not genetically related and have a low sequence homology [1], are absolutely stereospecific. We solved the crystal structure of the L-enzyme (6HLNO). It converts 6-hydroxy-*L*-nicotine to 6-hydroxy-N-methylmyosmine, which hydrolyzes spontaneously to a ketone [1].

Diffraction data from the shock-frozen protein structure (space group P432; a=164 Å) were measured using the CCD detector (MAR) on BW6. The native data extended to 1.95 Å (R-merge 4%; completeness 99.7%). Experimental phase determination was based on rapid derivatization immediately before data collection. First, a bromine derivative was prepared by a quick (10 s) soak with NaBr. MAD data were measured at the Br K-edge. The anomalous difference Patterson map could not be interpreted immediately. Therefore, a xenon derivative was prepared by diffusing Xe under high pressure (20 bar) into a protein crystal using commercial equipment (CryoXeSiter/MSC). A Xe derivative data set was used, in combination with solvent flattening, to derive SIRAS phases at 3.0 Å resolution. These phases permitted to locate the reference scatterers in the Br derivative. On this basis, MAD phases were calculated with MLPHARE at 2.3 Å resolution. The initial electron density maps were of such high quality that most of the polypeptide chain could be autotraced using wARP [2]. The missing amino acid residues and an FAD molecule were built manually with the program O. The model was refined with SHELXL-97 [3] to a current R-factor of 20% at 2.0 Å resolution.

The crystals contain one monomer of 425 residues (47 kD) per asymmetric unit. Fig. 1 shows a crystallographic dimer with the Xe and Br sites. Each monomer of the homodimeric enzyme contains a non-covalently bound FAD (Fig. 2), which is required as a cofactor. The flavin is located in the interior of the protein. A cavity is present in the immediate environment of the active site. In the native structure, this cavity is occupied by a water cluster.

[1] Schenk, S., et al. (1988), J. Mol. Biol. **284**, 1323-1339.
[2] Perrakis, Moris & Lamzin, (1999), Nat. Struct. Biol. **6**, 458-63.
[3] G.W. Sheldrick Göttingen University SHELXL-97 (1997)

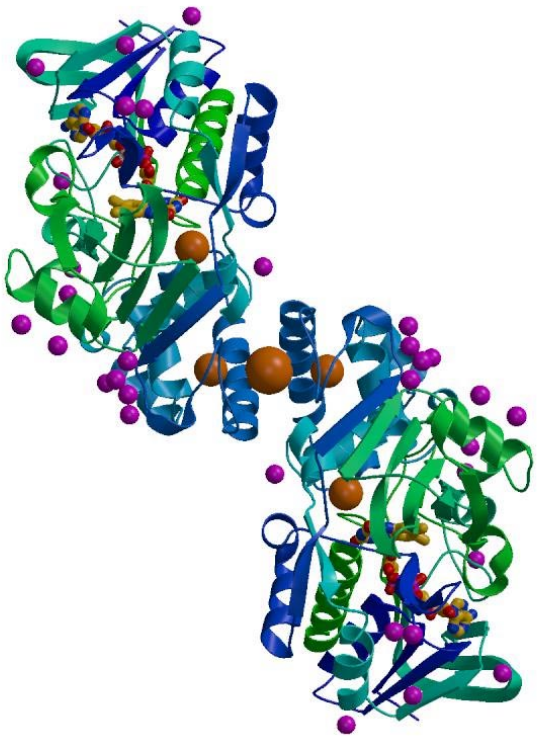

Fig. 1: Model of a crystallographic dimer with the Xe (orange) and Br sites (magenta)

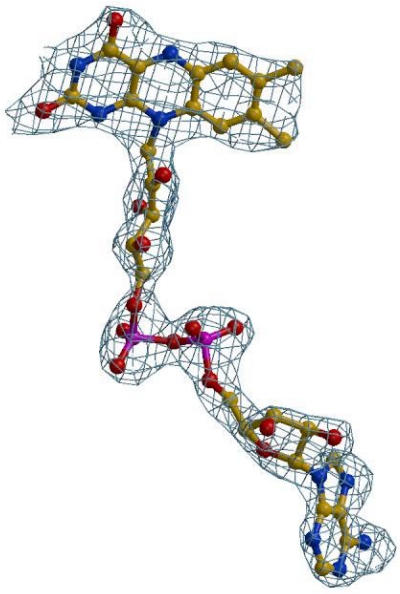

Fig. 2: Section of the initial electron density map showing the FAD