ESRF	**Experiment title: Diffraction Study of Haloalkane Dehalogenase LinB from *Sphingomonas paucimobilis* UT26 to Atomic Resolution**	**Experiment number:** LS-1473
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Report:

The haloalkane dehalogenase from *Sphingomonas paucimobilis* UT26 (LinB) is the enzyme involved in the degradation of the important environmental pollutant γ-hexachlorocyclohexane γ-HCH (γ-HCH). LinB catalyzes the conversion of 1,3,4,6-tetrachloro-1,4-cyclohexadiene to 2,5-dichloro-2,5-cyclohexadiene-1,4-diol via 2,4,5-trichloro-2,5-cyclohexane-1-ol during γ-HCH dechlorination by *Sp. paucimobilis* UT26. In addition to cyclic dienes, LinB also converts a broad range of halogenated alkanes and alkenes to their corresponding alcohols. The dehalogenation reaction is catalysed without oxygen or any other cofactor. The amino acid sequence of LinB showed significant similarity to two other haloalkane dehalogenases from *Xanthobacter autotrophicus* GJ10 and *Rhodococcus rhodochrous* NCIMB 13064. These three proteins belong to different specificity classes which are evolutionary optimized for conversion of different xenobiotic compounds. Determination of the structure of LinB is important for the study of the adaptation of microorganisms to the biodegradation of xenobiotic compounds at the molecular level. Understanding of the structural determinants of activity and specificity of

haloalkane dehalogenases will further facilitate the attempts to modify these enzymes for bioremediation purposes.

In experiment LS-1473, we have collected 1.45 Å data set with native LinB crystal and another 2.0Å data set with a complex of LinB with 1-chlorooctane, a good substrate of LinB. Both structures were successfully refined starting with the native LinB structure as input model using the programs REFMAC, CNS, ARP/wARP, SHELXL-97 and O.

The enzyme belongs to the α/β hydrolase family and contains a catalytic triad (Asp108, His272 and Glu132) in the lipase-like topological arrangement previously proposed from mutagenesis experiments. The LinB structure was compared with the structures of haloalkane dehalogenase from *Xanthobacter autotrophicus* and from *Rhodococcus* sp. and the structural features involved in the adaptation towards xenobiotic substrates were identified. The arrangement and composition of the α-helices in the cap domain results in the differences in the size and shape of the active site cavity and the entrance tunnel. This is the major determinant of the substrate specificity of this haloalkane dehalogenase.