Haloperoxidases catalyse the halogenation of organic compounds in the presence of halide ions and peroxides like H$_2$O$_2$. The bacterial haloperoxidases catalyse this redox reaction without the involvement of a known cofactor$^{-2}$. In order to elucidate the reaction mechanism recently the crystal structure of the cofactor-independent haloperoxidase CPO-A2 from the γ-chloro-tetrazolium-producing Streptomyces aureofaciens ATCC 10762 was solved by MIR$^3$. The overall structure of this haloperoxidase has been characterized as a α/β-hydrolase fold with a catalytic triad in the active center consisting of Ser 98, His 257 and Asp 228, but the exact mechanism of the enzyme remained still unclear. The halogenation reaction of these haloperoxidases requires the presence of organic acids like acetate or propionate. Therefore as reaction mechanism it was postulated, that acetate, activated by the nucleophile Ser 98, could be oxidised by peroxide to the peroxoacid in a first step, which then could oxidize the halide ion, probably with the involvement of as yet unidentified additional residues in the active center.

Crystals of haloperoxidase CPO-A2 were obtained at pH 8.0, where the enzyme shows only low residual activity$^3$. As part of this investigation of the reaction mechanism we determined the structures of the related haloperoxidase from Streptomyces lividans (CPO-L) both at pH 8.0 and at pH 6.0, the activity pH optimum. The role of Met99 in CPO-A2 was investigated with the CPO-A2 mutant Met99→Thr which is analogous in this position to CPO-L, but inactive. The structure of this mutant has also been determined and discussed their differences and the implications for the reaction mechanism.

CPO-A2 M99T from Streptomyces aureofaciens crystallizes from 1.8 M ammonium sulphate at pH 8.0. Crystals diffract to 1.5 Å resolution, belong to spacegroup I2 1 2 1 with a = 121.7 Å and contain one monomer in the asymmetric unit. CPO-L from Streptomycyes lividans TK64 crystallizes at pH 8.0 from 2.1 M ammonium sulphate in the space group P4 with a = 176.5 Å, c = 64.0 Å. Crystals diffract to 1.9 Å and contain one trimer per asymmetric unit. At pH 6.0 CPO-L crystals grow from 2.0 M ammonium sulphate in space group P2$_1$2$_1$2$_1$ and diffract to 2.6 Å. The structures have been solved by molecular replacement using AMORE and have been refined using X-PLOR.

When focusing on Heme-containing proteins, the three-dimensional structure is known for quite a few A large number of members of the Cytochrome c and Globin-like superfamilies, members of the superfamily of Heme-linked catalases and members from the superfamily of Heme-dependent peroxidases. However there is still one very important class of structures of heme-containing proteins that has been unknown until just recently. This is the class of plant peroxidases. Now however the structures of peanut peroxidase (Schuller D.J., Ban N., van Huystee, R.B., McPherson A. & Poulos T.L. (1996) Structure, in press), barley peroxidase (Henriksen A., Welinder K.G. & Gajhede M. in preparation) and recombinant horseradish peroxidase C (HRPC$^*$) (Gajhede M., Schuller D.J, Henriksen A., Smith A.T. & Poulos T.L. in preparation) have been solved. The structure of HRPC$^*$ has been solved by molecular replacement, using the peanut peroxidase structure as a model. The structure has been solved in two crystal forms: with and without the substrate benzene hydroxamic acid bound in the active site. A detailed analysis of the two structures will be presented.