Rat peroxosomal multifunctional enzyme type 1 (perMFE-1) is a monomeric protein that catalyses the second and the third reaction in the $\beta$-oxidation of fatty acyl-CoA molecules in separate active sites. perMFE-1 possesses enzyme activities for the hydratase-1/isomerases (H1/I) and (3S)-hydroxyacyl-CoA dehydrogenases (HAD) reactions. The H/I reaction is located in the N-terminal part of the polypeptide (domain A) and the HAD activity (domains C and D) is between the B and E domains, which have a structural role and participate in intracellular targeting of the protein [1].

Crystallographic studies have been initiated to resolve the structure of the perMFE-1. The results obtained by crystallographic methods are used together with kinetic data on the wild type and variant enzymes to further elucidate the mechanisms of catalysis and substrate and cofactor trafficking from one active site to the other.

The HAD domain together with the E domain has been crystallised [2]. The crystallisation conditions consisted of 0.2M sodium citrate, pH 5.6, 20mM ammonium acetate and 27% poly ethylene glycol 4000. Crystals reached the size of 0.35 x 0.2 x 0.2 mm in 30d. Data has been collected from unliganded crystals to 2.45Å resolution using a rotating anode X-ray source. The unliganded crystal has primitive tetragonal space group with unit cell parameters $a=b=125.9$, $c=60.2\text{Å}$. Molecular replacement method has not been successful and thus trials for finding suitable heavy metal derivatives have been initiated. Data has been collected at EMBL Hamburg X13 and BW7A beamlines to 2.5-3.5Å resolution for isomorphous replacement phasing but the initial phases have not been sufficiently good for calculating interpretable electron density maps. MAD data of SeMet labelled liganded crystals have been collected to 3.5Å resolution but Se sites could not be found, possibly due to poor labelling of the Met residues (30% labelled as analysed by mass spectrometry). The liganded SeMet labelled crystal has primitive tetragonal space group with unit cell parameters $a=b=94.0$, $c=59.3\text{Å}$.

Figure 1: Diffraction image of an liganded Br derivative crystal recorded at the beamline BW7A. The edge of the picture corresponds to 2.7Å resolution.

References