ESRF	**Experiment title:** Structure elucidation of mutants from the Staphylococcus epidermidis amidase AmiE	**Experiment number**: MX 750
Beamline: ID14-1	**Date of experiment**: from: 28 April 2008 to: 28 April 2008	**Date of report**: 23.10.2008
Shifts: 2	**Local contact(s)**: Mr Gianluca CIOCI	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Mr Bernhard PAETZOLD University of Tuebingen*
Mr Sebastian ZOLL University of Tuebingen*
Mr Nicolaus Schmitz University of Tuebingen*

Report:

During the experiment MX750 we could collect various datasets. These included a high resolution dataset of an inactive mutant. We also measured mutant and wt crystals soaked or cocrystallized with two putative ligands.

We were successful to solve the structure of the mutant to in the uncomplexed form using molecular replacement. The structures allowed us to gain insights in the structural features underlying the inactivity of the two mutants. This significantly improved our understanding of the catalytic mechanism of the AmiE. The evaluation of the structure allowed us to understand why another mutant prepared is still reluctant to crystallize. This was only possible because of the high resolution obtained at the ESRF.

Unfortunately we could not collect a dataset for the ligand bound structure. Neither the soaked crystals nor the many crystals resulting from cocrystalization had the ligand bound. The crystals in the soaking experiments suffered after prolonged exposure to the soaking solution. We therefore concluded that the ligand might bind to the protein and affect the crystal packing. During MX750 we measured crystals that were soaked for the maximum soaking time while miminzing suffering of the crystal. Merging the dataset of the soaked crystals with the native dataset showed no difference.

Tab. 1 Data collection and refinement statistics of the inactive mutant. Values in parentheses are for the highest-resolution shell.

	D179-MDP
Data collection	
Space group	$P2_12_12_1$ (No.19)
Cell dimensions	
a, *b*, *c* (Å)	38.87, 57.95, 98.06
α, β, γ (°)	90, 90, 90
Resolution (Å)	20–1.75 (1.80-1.75)
R_{merge}	5.9 (34.1)
R_{meas}	6.9 (39.3)
$I / \sigma I$	20 (4.57)
Completeness (%)	99.4 (99.6)
Redundancy	3.52 (4)
Refinement	
Resolution (Å)	20-1.75
No. reflections	21679
R_{work} / R_{free}	19.59/24.21
No. atoms	
Protein	1685
Zinc ion	3
Water	326
B-factors (Å^2)	13.57
Protein (Å^2)	13.42
Water (Å^2)	24.80
R.m.s. deviations	
Bond lengths (Å)	0.014
Bond angles (°)	1.274

We had various crystals resulting from cocrystalization. We collected and refined about 6 datsets to ensure that no ligand was in the active site cleft. Unfortunately no clear electron density for the ligand was visible.
We are currently trying to synthesize new custom made ligands which have a higher affinity. We are also working on imrpovements of our soaking and cocrystalization protocols, based on results from docking experiments. The results we have obtained up to now will be published as soon as we obtain the ligand bound structure.