ESRF	**Experiment title:** Porcine acylaminoacyl peptidase	**Experiment number**: MX 1602
Beamline: ID23-2	**Date of experiment**: from:06/02/2014 to: 07/02/2014	**Date of report**:
Shifts: 1	**Local contact(s)**: Dr. Alexander Popov	*Received at ESRF:*

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Report:

Project Goals

While archaeal orthologues Acylaminoacyl peptidase (AAP) are relatively easy to crystallize, solving the structure of mammalian AAP was not yet successful. Our previous trials to solve its structure failed because 1) of seriously anisotropic diffraction of the crystals 2) the crystals were thin plates and usually twinned. The aim of the project is to test and collect data from several crystals grown with different additives and find crystal(s) to lessen twin fractions, and also improve resolution. We intended to use microfocus beam for data collection, finding parts of the crystals with less twin fractions and improved diffraction properties.

We tested crystals of a truncated form of dUTPase, transglutaminase 2, human S100A4 / human Annexin A2 complex and beta-D-xylosidase.

Project achievements

We screened several crystals of porcine AAP using mesh scan to find crystals/ crystal regions with improved diffraction characteristics (and sort out twinned crystals). We collected data sets from 3 crystals, all of them containing AAP complexed with covalent inhibitor (starting resolution: 2.9 Å, resolution in worse orientation: 5-6 Å). Crystals containing heavy atom derivatives diffracted very weakly (10-30 Å). The phase problem could not be solved using these data sets.
Since the data collection period, a new crystal form was found diffracting to higher resolutions allowing to solve the phase problem by molecular replacement. Crystals of the second crystal form tend to be merohedrally twinned, while the first crystal form studied during MX 1602 experimental session show single plate crystals difficult to separate. This fact and the difference in the dimensions of the asymmetric units suggest that the

dominant organizations and/or relative positions of the AAP monomers within their tetramer may be different (which occurred in AAPs from different species) in the two crystal forms. Thus the refined structure of the second crystal form will be used to solve the structure for data sets collected during experimental session MX 1602, and the structures of the two crystal forms will be compared for finding large scale differences detectable at lower resolution.

A high resolution X-ray diffraction dataset (1.3 Å) was collected from a single crystal containing an active site truncated Mycobacterium tuberculosis dUTPase structure in complex with dUPnPP non-hydrolysable substrate analogue. After structure solution, coordinates as well as structure factors were deposited at the PDB with accession code 5ECT and they are on hold until publication. The structural insights of this mutant enable the in-depth understanding of the role of critical enzyme segments in catalysis. A manuscript has already been composed including these results and is close to submission.

A dataset of human S100A4 / human Annexin A2 complex was collected to 2.7 Å resolution. Unfortunately the crystal contained only the apo (uncomplexed)protein.

Transglutaminase 2 and beta-D-xylosidase crystals were tested, but they showed very weak diffraction.