ESRF	**Experiment title:** Structural basis of tetramer formation of acylaminoacyl peptidase	**Experiment number**: 14-U- 831
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Shifts: 3	**Local contact(s)**: Hassan Belrhali	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Veronika Harmat*

Balázs Hornung

Report:

Acylaminoacyl peptidase (AAP), a member of the prolyl oligopeptidase family removes acylated amino acids from the N-terminus of oligopeptides. It is involved in the immune response in the gastrointestinal tract. AAP is a potential target for cognitive enhancing drugs and for organophosphorus compounds, and it is linked with diverse carcinomas.

Mammalian AAP is a tetramer, for which crystal structure determination was unsuccessful so far. The only AAP with known structure, is the dimeric enzyme form Aeropyrum pernix K1. To explain the importance of oligomerization in the catalytic action of mammalian AAP, we chose as model AAP from Pyrococcus horikoshii OT3, which is a tetramer.

The aim of the project was to collect MAD datasets and a native dataset with improved resolution (2.0 Å resolution at home source).

We collected peak and inflection wavelength datasets of a Pt derivative (high energy remote dataset was collected at DESY beamline X11). A dataset was collected from a crystal co-crystallized with an inhibitor. Space group H32, a,b=183.0 Å, c=144.6 Å. Co-crystallization of AAP with a substrate resulted a second crystal form (space group $P2_12_12_1$, a=180.7 Å, b=181.0 Å, c=271.9 Å, resolution 2.8 Å) A dataset from a Pt derivative of this crystal form was also collected (resolution 4 Å).

The phase problem for the first crystal form was solved by MIRAS method using datasets collected from Pt U and I derivative crystals (further data were collected at our home source). Model building and refinement are in progress.

Initial analysis of the structure reveals that the two-domain structure is more open than in most members of the family. This conformation of the molecule allows the substrate to access the active site. The AAP

molecules form crystallographic trimers with tightly packed beta-propeller domains. Contacts between the trimers are formed through the hydrolase domains.

The crystals of the second crystal form show considerably weaker diffraction. The volume of the asymmetric unit is nearly ten times greater than that of the previous crystal form. Our attempts to solve the structure of this crystal form were not successful so far. The Pt derivative showed only weak signal. However, the importance of this form is underlined by the fact that the self rotation function indicates non-crystallographic symmetry with four-fold rotation component. This suggests that the crystals contain tetramers corresponding to the natural oligomerization state. Data collection from further derivatives is needed to solve the structure.