ESRF	**Experiment title:** Structural basis of tetramer formation of acylaminoacyl peptidase	**Experiment number**: 14-U-831
Beamline: BM14U	**Date of experiment**: from: 29/06/2006 to: 30/06/2006	**Date of report**: 25/11/2013
Shifts: 3	**Local contact(s)**: Hassan Belrhali	*Received at ESRF:*

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Report:

Acylaminoacyl peptidase (AAP), a member of the prolyl oligopeptidase family removes acylated amino acids from the N-terminus of oligopeptides. Its function is still not clear in all details. It has been shown to act in concert with and exert regulation over the proteasome while also being implicated in renal and small cell carcinoma, and it is also referred to as a potential target of cognitive enhancing drugs. Mammalian AAP is a tetramer, for which crystal structure determination was unsuccessful so far. The aim of the project was to solve the structure of an archeal AAP (AAP from Pyrococcus horikoshii OT3, PhAAP), which previously thought to be tetrameric.
We collected at beamline BM14U peak and inflection wavelength datasets of a Pt derivative of PhAAP which were used to solve the structure together with datasets collected at home source and at DESY beamline X11. A native dataset from a second crystal form was also collected at beamline BM14U, the structure of which was also determined. (Our study was published in J. Biol. Chem.)

Though as a result of our study, PhAAP was proven to be non-tetrameric, and as such, not a good model for studying the domain- and monomer organization of the tetrameric mammal orthologues; the conclusions of the structural analysis were found to be generalizable in the prolyl oligopeptidase family.

Main Results:

- We solved the structure of PhAAP, and proved that it is hexameric (also supported by later size exclusion chromatography experiments).
- Comparison of the structures of the two crystal forms suggest a rigid structure with fixed channel system connecting the active sites of the monomers and the solvent.
- We concluded that the substrate access and substrate size-selection mechanism is achieved by self-compartmentalization.
- We suggested that the different strategies of substrate access and substrate size-selection within the prolyl oligopeptidase family are related to different ways of shielding an amylogenic β-edge of the enzymes.

Reference:
D. K. Menyhárd, A. Kiss-Szemán, É. Tichy-Rács, B. Hornung, K. Rádi, Z. Szeltner, K. Domokos, I. Szamosi, G. Náray-Szabó, L. Polgár, V. Harmat:
A Self-compartmentalizing Hexamer Serine Protease from Pyrococcus Horikoshii. Substrate Selection Achieved Through Mmultimerization .
J. Biol. Chem, **288**: 17884–17894, 2013

Abstract:
Oligopeptidases impose a size limitation on their substrates, the mechanism of which has long been under debate. Here we present the structure of a hexameric serine protease, an oligopeptidase from Pyrococcus horikoshii (PhAAP), revealing a complex, self-compartmentalized inner space, where substrates may access the monomer active sites passing through a double-gated "check-in" system, first passing through a pore on the hexamer surface and then turning to enter through an even smaller opening at the monomers' domain interface. This substrate screening strategy is unique within the family. We found that among oligopeptidases, a residue of the catalytic apparatus is positioned near an amylogenic β-edge,which needs to be protected to prevent aggregation, and we found that different oligopeptidases use different strategies to achieve such an end. We propose that self-assembly within the family results in characteristically different substrate selection mechanisms coupled to different multimerization states.