EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Structural analysis of a member of a SUMO protease family (SENP6) and a novel tri-domain class of carboxypeptidases.	**Experiment number**: MX-1354
Beamline: ID23-1	**Date of experiment**: from: 17 Nov 2011 to: 18 Nov 2011	**Date of report**: 8 May 2013
Shifts: 2	**Local contact(s)**: Daniele De sanctis	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

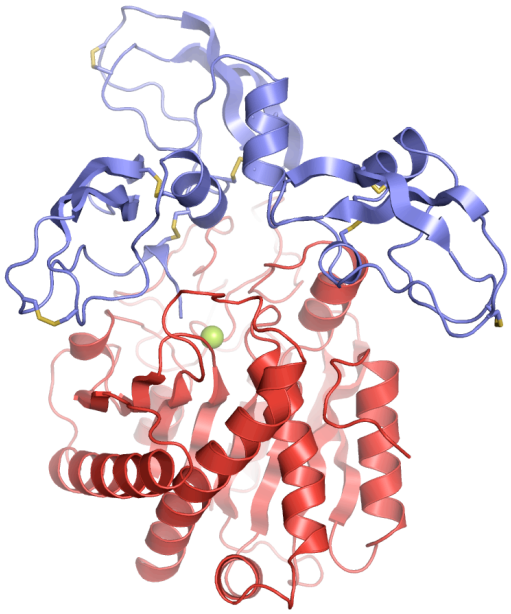
Two new publications were derived from these ESRF trips:

Alonso-del-Rivero, M., Reytor, M.L., Trejo, S.A., Chavez, M.A., Aviles, F.X., and **Reverter, D.** (2013). A novel and non-canonical mechanism of carboxypeptidase inhibition revealed by the crystal structure of the tri-Kunitz SmCI in complex with human CPA4. **Structure** (in press).

ABSTRACT: The crystal structure of SmCI (*Sabellastarte magnifica* Carboxypeptidase Inhibitor) has been determined in complex with human carboxypeptidase A4 (hCPA4). SmCI is composed by three BPTI/Kunitz domains, each one displaying high structural homology and functionality with serine protease inhibitors. Moreover, SmCI possesses a distinctive capability to inhibit metallo-carboxypeptidases, constituting the first example of a bi-functional metallocarboxy- and serine-protease inhibitor. The structure of the 1:1 complex of SmCI with hCPA4 reveals a non-canonical mechanism of carboxypeptidase inhibition, which surprisingly occurs mainly via the N-terminal segment, which penetrates into the active site groove of the enzyme. Mutagenesis and biochemical analysis confirm the major role of the N-terminal segment in the inhibition of carboxypeptidases. SmCI constitutes the only example of a tri-Kunitz serine protease inhibitor adapted to inhibit metallo-carboxypeptidases and discloses an unusual mechanism of inhibition by the N-terminal segment, emulating the “classical” C-terminal substrate-like inhibition.

Diffraction data were recorded from cryo-cooled crystals (100K) at Grenoble beamline ID23-1.

Accession codes – Protein Data Bank: Coordinates and structure factors were deposited in the PDBe data with accession code 4BD9



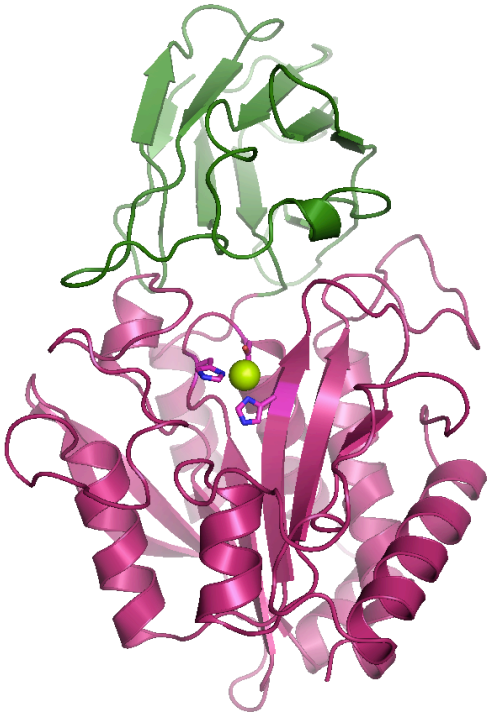
Structure of SmCI (blue) in complex with human carboxypeptidase A4 (red)

Otero, A., Rodríguez de la Vega, M., Tanco, S., Lorenzo, J., Avilés F.X. and **Reverter, D.** (2012). The novel structure of a cytosolic M14 metallo-carboxypeptidase (CCP) from *Pseudomonas aeruginosa*. A model for mammalian CCPs. **FASEB J.** 26(9): 3754-3764.

ABSTRACT: PaCCP is a metallo-carboxypeptidase (MCP) from *Pseudomonas aeruginosa,* which belongs to the bacterial family of carboxypeptidases that are homologous to the recently discovered subfamily of human non-secretory cytosolic carboxypeptidases (CCPs). CCPs are intracellular peptidases involved, among other roles, in the post-translational modifications of tubulin. Here we report the crystal structure of PaCCP at high resolution (1.6 Å). Its 375 residues are folded in a novel β-sandwich N-terminal domain followed by the classical carboxypeptidase α/β-hydrolase domain, this one in a shorter and more compact form. The former is unique in the MCP family and does not have sequential or structural homology with other domains that are usually flanking the latter, like the pro-domain of the M14A subfamily or the C-terminal transthyretin/prealbumin-like domains of the M14B subfamily. Unexpectedly, PaCCP does not display activity against small carboxypeptidase substrates. Structural results derived from co-crystallization with well-known inhibitors of MCPs indicate that the enzyme might only possess C-terminal hydrolase activity against cellular substrates of particular specificity and/or when undergoes structural rearrangements. The derived PaCCP structure allows a first structural insight into the more complex mammalian CCP subfamily.

Diffraction data were recorded from cryo-cooled crystals (100K) at Grenoble beamline ID23-2.

Accession codes. - Coordinates and structure factors from the three structures were deposited in the PDB data with accession codes 4a37, 4a38 and 4a39.



Structure of *Pseudomonas aureginosa* carboxypeptidase. In green is depicted the N-terminal domain, and in red the carboxypeptidase domain. Zinc is represented as a yellow solid sphere.