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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 using the **Electronic Report Submission Application:**

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Reports on experiments relating to long term projects

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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:** ***Crystal polymorphism a crystal quality in Abl-SH3 domains mutants and the implications in the accuracy of solvent determination***	**Experiment number**: MX-739
Beamline: BM16	**Date of experiment**: From: 03/03/2008 to: 04/03/2008	**Date of report**: 1/07/2008
Shifts: 3	**Local contact(s)**: Ana LABRADOR /Fox GAVIN	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Ana CAMARA-ARTIGAS, Dpto. Quimica Fisica, Bioquimica y Quimica Inorganica, Universidad de Almeria, SPAIN

Patricia PALENZUELA, Dpto. Quimica Fisica, Bioquimica y Quimica Inorganica, Universidad de Almeria, SPAIN

Jose A. GAVIRA, Laboratorio de Estudios Cristalográficos-IACT, Granada, Spain

Report:

In MX-739 experiment we were able to measure several datasets of the Abl SH3 domain, Src SH3 domain, α-spectrin SH3 domain and the TSG-101 domain. All these protein domains are characterized by the interaction with proline rich peptides. The porpouse of these measurements is to improve the data previously obtained in a in-house difractometer, because the main target of our research is to model water molecules with high accuracy. These data are needed to check the hypothesis of the presence of water molecules in the interface of the binding site, which can explain the thermodynamic signature of the binding isotherms obtained by means of ITC [1]. We have already high resolution data of the N114A Abl-SH3 domain, and this mutant show a change in the space group which is pH dependent. We have crystallized the WT protein of the Abl SH3 domain at several pHs, and we have measured these crystals at room temperature and at criogenic temperatures. We have used two different crystallization setup in order to find the better conditions to improve the crystal quality: crystals obtained in a vapour diffusion setup in the Almeria University lab, while crystals obtained using the contradiffusion technique were obtained in the Laboratorio de Estudios Cristalograficos. Unfortunately the crystals grown using the contradiffusion technique have a space group and unit cell unusual for the Abl SH3 domain, and the values are quite similar to those obtained for the the α-spectrin SH3 domain. It makes us suspect that these crystals belong to the α-spectrin SH3 domain instead of the Abl SH3 domain. As finally was checked by solving the structure by molecular replacement, using the WT α-spectrin SH3 domain coordinates (PDB code 1SHG) these crystals belongs to this SH3 domain.

pH	Crystallization setup	Space group	Unit cell	Resolution
3	Vapour diffusion	$P3_212$	49.293 49.293 44.945 90 90 120	2.7
5	Vapour diffusion	$P3_212$	50.288 50.288 45.167 90 90 120	1.6
7	Vapour diffusion	$P3_212$	50.401 50.401 45.362 90 90 120	1.9
9	Capilar	$P2_12_12_1$	34.459 42.468 50.031 90 90 90	1.55
5	Capilar	$P2_12_12_1$	32.720 42.336 50.335 90 90 90	1.93

We also measured one small crystal (<50μm) of the Src SH3 domain. There is not crystallographic structure of this SH3 domain, and it is very important for us to model the solvent in other SH3 domains to check the universal pattern of the binding of the polyproline peptides to these domain. This crystal diffracts up to 1.6 Å resolution and seems to belongs to the Laue group P3.

Crystals of the mutant R21D of the α-spectrin SH3 domain was also mesured to improve the quality of the data. We have in house data up to 1.4 Å, and we have measured a crystal grown under oil which diffracts at 1.1 Å. Our interest in to increase the resolution of this data, beside our major target in to improve the water modelling, is to study the basis of this unusual polymorphims. Up to date, all the crystals measured of the α-spectrin SH3 domain belongs to the orthorhombic space group $P2_12_12_1$.

Finally, we have measured several crystals of the TSG-101 domain. Recently, we have solve the crystallographic structure of this domain [2]. Our goal in these measurements was to improve the in-house data of the complex between the nonapeptide ILPTAPPEY belonging to the Ebola virus, which best crystals diffracts at 3 Å. For this experiment we have growth crystals of almost 1mm of final size by means of seedeing techniques. Besides the high size of the crystals, we measured 5 cyrstals and the best diffracts at only 2 Å resolution. However this results is a considerable improvement, as allows us to model the solvent. The crystals belongs to the space group I4, with a unit cell of 105.55 105.55 75.23. Besides we manage to get a complete set of data of the complex of the TSG-101 domain with the decapeptide of the Leukemia-c virus YVEPTAPQVL. In this case the crystals belongs to the Laue group P3 with unit cell 168.971 168.971 38.231, but the best crystals only diffracts up to 3.5 Å. We need to improve these crystals in order to obtain better quality data to be able to model the solvent in these complex.

References

1. **Palencia, A., et al., *Thermodynamic dissection of the binding energetics of proline-rich peptides to the Abl-SH3 domain: implications for rational ligand design.* J Mol Biol, 2004. 336(2): p. 527-37.**
2. **Palencia, A., et al., *Structure of human TSG101 UEV domain.* Acta Crystallogr D Biol Crystallogr, 2006. 62(Pt 4): p. 458-64.**