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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 supporting requests for additional beam time

Reports can now 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.

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Reports on experiments relating to long term projects

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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.

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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: Intertwined dimeric structures of the SH3 domain of the c-Src tyrosine kinase.**	**Experiment number**: MX-1225
Beamline: ID14-4	**Date of experiment**: from: 28/01/2011 to: 29/01/2011 from: 04/04/2011 to: 05/04/2011	**Date of report**: 19/08/2011
Shifts:2	**Local contact(s)**: **Andres PALENCIA**	*Received at ESRF:*

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SCIENTIFIC BACKGROUND :

SH3 (Src Homology 3) domains are widespread proline-rich recognition modules, found in many different proteins, in diverse numbers and combinations. These domains are implicated in deregulated signaling pathways during cancer development and are also associated to other pathologies such as AIDS, osteoporosis, or inflammatory processes. Typically, SH3 domains act as docking sites for the recruitment of substrates and the formation of supra-molecular complexes leading to the enzymatic modification of some of their components. However, in some cases, they also play an essential role in the regulation of the enzymatic activity of the proteins that contain these domains. This is the case of the Src family of tyrosine kinases, which comprises a subclass of membrane-associated non-receptor tyrosine kinases involved in cellular signal transduction pathways, and are frequently over-expressed and/or aberrantly activated in a variety of cancers. Although these kinases have been postulated to perform redundant functions in the cell, their implication in tumour development is different and there is an increasing body of evidence for specificity in signalling, in some instances determined by interactions mediated by their SH3 domains.

GOAL:

Our research group interest is focus in the determination of high resolution crystallographic structures of the SH3 domains and their complexes in order to characterize the molecular components of binding specificity between these domains. Besides, in the case of the c-Src-SH3 domain and as results of previous measurements in the BM16 beam line at the ESRF, we have solved recently the structure of this domain[1]. In this structure the principal feature is that the asymmetric unit contains two c-Src-SH3 molecules associated as an intertwined dimer. Our goal in these measurements will be to determine the structures of several mutants of this domain to study the molecular basis of the domain swapping process.

EXPERIMENT AND RESULTS:

In MX-1225 experiment we were able to measure several datasets for some mutants of the c-Src SH3 domain and the complexes formed by the mutants T98E and T98D of the c-Src SH3 domain with the ligand APP12, the complex of the mutant T98E with the ligand VSL12 (see table 1). These crystals were grown, as well as the wild type, in the presence of PEG of low molecular weight (PEG200 or PEG300). The datasets obtained indicate that all the crystals of the free form belong to the space group $P6_5$ with a unit cell parameters very similar to the observed for the c-Src-SH3 domain [1]. It makes us suspect that, as well as c-Src-SH3 domain, the intertwined dimer might be induced by the presence of PEG, as it was confirmed in the structure solve from these data. However the crystals of the complex

forms belongs to a different space group, $P3_221$, and in this case the presence of the high affinity polyproline ligand seems to displace the equilibrium to a monomeric form of the domain.

We also have measured crystals from other SH3 domains complexed with poly-proline rich ligands. In the case of the Abl-SH3 domain, even the crystallization conditions are very alike to those where the intertwinned Src-SH3 domain crystals growth, not intertwinned structures have been described for this domain (Table 1). We are interested in to characterize these complex structures, as we are working also with quimeric proteins where the loops of the Src-SH3 domain have been introduced into the Abl-SH3 structure, to study if the determinans of the partial unfolding are un the core of the folding of the protein or is enable by the composition of the loops. Besides, we have recently reported the importance of the role play by water molecules in the binding of the proline-rich sequences by the Abl-SH3 domain [2], and the high resolution structures of the crystals mesured in this experiment can help to better understand this role.

The crystals measured during this experiment are reported in Table 1.

We are thankful to Dr. Andrés Palencia for his strong support during the data collection, especially for his invaluable help during the serious incident that happens at the data collection in January.

Table 1

Protein	Space group	Unit Cell	Resolution (Å)
Src-SH3-S94A	$P6_5$	46.58, 46.58, 127.01	1.95
Src-SH3-T96G	$P6_5$	46.76, 46.76, 128.77	2.8
Src-SH3-N114S	$P6_5$	46.95, 46.95, 126.71	1.9
Src-SH3-R128K	$P6_5$	46.57, 46.57, 127.25	2.4
Src-SH3-R128Q	$P6_5$	47.01, 47.01, 126.56	2.0
Src-SH3-T98E/VSL12	$P3_221$	37.41, 37.41, 85.65	1.05
Src-SH3-T98E/APP12	$P3_121$	31.59, 31.59, 106.71	1.30
Src-SH3-T98D/APP12	$P3_121$		1.25
Abl -SH3-WT/P0	$P3_221$	86.42, 86.42, 45.16	1.01
Abl -SH3-WT/P7	$P3_221$	86.58, 86.58, 45.27	1.7
Abl-SH3-N114A/P0	$P3_221$	86.40, 86.40, 44.95	1.5
Abl-SH3-N114A/P7	$P3_212$	50.07, 50.07, 45.00	1.6
Abl-SH3-N114A/P17	$P3_221$	86.35, 86.35, 45.1	
PDZ3-PSD95	$P3_112$	61.95, 61.95, 228.15	1.6
BPE	H3	187.08, 187.08, 59.21	1.7
Fyn-SH3/NS5A (MAD)	$P4_12_12$	51.28, 51.28, 184.34	1.8

1. Camara-Artigas, A., Martin-Garcia, J.M., Morel, B., Ruiz-Sanz, J., and Luque, I. (2009). Intertwined dimeric structure for the SH3 domain of the c-Src tyrosine kinase induced by polyethylene glycol binding. FEBS Lett *583*, 749-753.
2. Palencia, A., Camara-Artigas, A., Pisabarro, M.T., Martinez, J.C., and Luque, I. (2010). Role of interfacial water molecules in proline-rich ligand recognition by the Src homology 3 domain of Abl. J Biol Chem *285*, 2823-2833.