ESRF	**Experiment title:** STRUCTURE OF THREE FRAGMENTS OF THE PLAKIN DOMAIN OF PLECTIN	**Experiment number**: MX1075
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Report:

Experiment objective.

The aim of this experiment was the collection of high resolution data from crystals of three fragments of plectin. We denote these fragments as Ple40, Ple44, and Ple56.

Background

Plakins are a family of high molecular weight proteins that interconnect elements of the cytoskeleton and tether them to membrane-associated complexes, hence, they are also known as cytolinkers. Plectin is has a tripartite structure consisting of N- and C-terminal regions separated by a central rod domain. This overall domain organization is also observed in other members of the plakin family. We had previously elucidated the crystal structures of the actin binding domain (1) and the first pair of spectrin repeats (2) of plectin. Here, we aimed at elucidating the crystal structures of additional regions of plectin.

Results

The Ple56 fragment corresponds to the forth and fifth spectrin repeats (SR4-SR5) and the SH3 domain of the plakin domain of plectin. 24 crystals of this fragment were analyzed in this experiment. The best diffracting crystals allowed for the collection of a dataset to maximum resolution of ~3.10 Å (Table 1). At the time of this experiment, these were the highest resolution data available for the crystals of this fragment. Later on, we have collected a dataset to 2.95 Å resolution using a rotating anode generator and a crystal subjected to post-crystallization dehydration (it is possible that the improvement of the diffraction observed could be due to the serendipitous finding of an unusually good-diffracting crystal). That 2.95 Å

dataset was eventually used to solve and refine the crystal structure of this region, which was deposited in the PDB under the code 3PE0 (3).

Table 1. Data collection statistics of the best-diffracting Ple56 crystal.	
Space group	$P2_12_12_1$
Unit cell	a= 72.2 Å b=107.8 Å c=112.1 Å α=β=γ=90º
Wavelength (Å)	0.933
Resolution (Å)	3.10 (3.27 - 3.10)[a]
Unique reflections	16423
Multiplicity	14.5 (14.9)[a]
Completeness (%)	99.9 (99.9)[a]
Rmeas (%)	5.9 (65.3)[a]
<<I>/<σI>>	30.1 (4.6)[a]
[a] data in parenthesis correspond to the higher resolution shell.	

26 crystals of the Ple44 fragment were analyzed during this experiment. Diffraction from these crystals shows significant anisotropy. Out of the 26 crystals, four diffracted to a resolution higher than 4.0 Å, and datasets were collected from them. Of those, the best diffracting crystal yielded a dataset to ~3.5 Å in the best direction (Table 2). Crystals belong to the space group C2 with unit cell dimensions a=75.3 Å, b=90.8 Å, c=154.7 Å and β=99.2º, and they contain two molecules in the asymmetric unit.

Table 2. Data collection statistics of the best-diffracting Ple44 crystal.	
Space group	C2
Unit cell	a= 75.3 Å b=90.8 Å c=154.7 Å α=90º β=99.2º γ=90º
Wavelength (Å)	0.933
Resolution (Å)	3.5 (3.7 - 3.5)[a]
Unique reflections	11322
Multiplicity	7.2 (6.9)[a]
Completeness (%)	86.8 (62.4)[a]
Rmeas (%)	8.4 (75.9)[a]
<<I>/<σI>>	13.5 (3.2)[a]
[a] data in parenthesis correspond to the higher resolution shell.	

The structure of the Ple44 has been solved by molecular replacement and has been refined against the aforementioned dataset. A region of a simulated-annealing omit map after B-factor sharpening and two-fold NCS average is shown in figure 1.

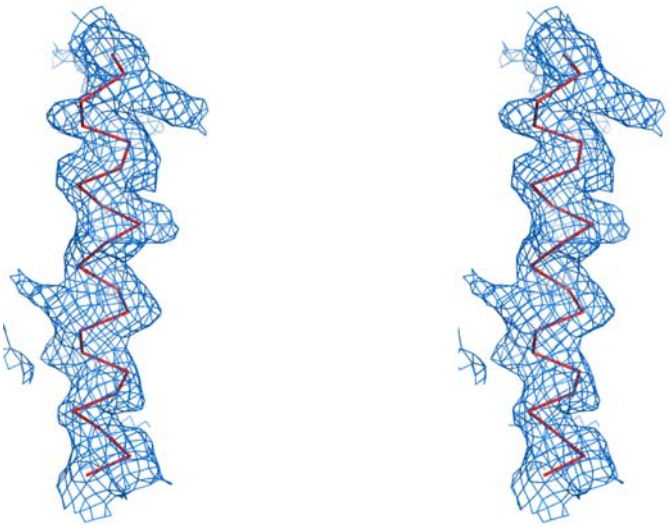

Figure 1. **Simulated-annealing (SA) omit map of a region of the Ple44 structure calculated with data collected during this experiment**. Stereo representation of a region of the map corresponding to a α-helix (shown in red as a Cα trace) which was removed from the model before doing a cycle of SA-based refinement. The map was sharpened by applying a -100 $Å^2$ B factor, and it was averaged using the two-fold NCS.

Crystals of the Ple40 fragment, which corresponds to the third and fourth spectrin repeat (SR3-SR4) were not analyzed due to the limitations in the number of crystals that can be transported in our dry-shipper and the priority of the pPle56 and Ple44 crystals.

Incidents

During the experiment we suffer from problems with the cryo-system which lead to the lost of four crystals.

References

1. Garcia-Alvarez, B., Bobkov, A., Sonnenberg, A., and de Pereda, J. M. (2003) *Structure* **11**, 615-625
2. Sonnenberg, A., Rojas, A. M., and de Pereda, J. M. (2007) *J Mol Biol* **368**, 1379-1391
3. Ortega, E., Buey, R. M., Sonnenberg, A., and de Pereda, J. M. (2011) *J Biol Chem* **286**, 12429-12438