| ESRF | **Experiment title: Macromolecular Crystallography at South-East Andalusia** | **Experiment number**: MX-1541 |
|---|---|---|
| **Beamline**: BM30A | **Date of experiment**: From: 30 November 2013 to: 01 December 2013 | **Date of report**: 04/02/14 |
| **Shifts:** 3 | **Local contact(s)**: Michel Pirocchi ( email: pirocchi@esrf.fr ) | *Received at ESRF:* |

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**Partial Report of Mx/1541 BM30A (30-11-2013 / 01-12-2013):**

In the beamtime allocation on November 30th, we brought to the ERSF almost 100 samples. Unfortunately, we did not get as many results as initially expected (see Tables 1& 2) of the target proteins. First, we realized that the content of both dewars, coming from Almeria and Granada, was not preserved as expected. In fact, all crystals from the dewar coming from Almeria did not diffracted X-ray at all. Similar situation was observed from the dewar coming from Granada. Even though some crystal diffracted X-ray to a middle resolution. On top of that, the detector suffered some type of fatigue and started showing lines on the images at around 5:30 am. This was not a big issue since we were already testing for diffraction more than collecting data at all. Below we resume the samples bring to the ESRF and the expectation.

Crystal from the dewar coming from Almeria belongs to several subjects:

**i) Protein miss-folding and disease.** From this subject we try to measure several different proteins:

***c-****Src-SH3 domain*. Crystal of a double mutant H122R/Q128E in complex with a proline rich motif of the NS5A (Non-Structural 5A protein of the Hepatitis C virus). The crystals of the free protein diffracts a near atomic resolution (data collected at XALOC of the ALBA synchrotron, Barcelona, Spain).

*α-spectrin SH3 domain.* We have previously collected data at ~1Å in the beamline XALOC of the ALBA synchrotron (Barcelona, Spain). In this beamtime allocation we planned to complete data to characterize some modified residues by collecting data at atomic resolution.

**ii) Proline rich sequences (PRMs) binding domains.** We manage to obtain crystals of the first WW domain of the YAP65. In October 2013, we collected data at 1.5 Å from very tiny crystals (<20μm) in the beamline XALOC of the ALBA synchrotron (Barcelona, Spain). The crystals diffraction was very good. In this beamtime allocation, we tried to improve the data to obtain the phases to solve the structure. Additionally we have crystallized other WW domains of the YAP65: WW2 and the tandem WW1-WW2, co-crystallized with and without proline rich motifs.

**iii) HIV vaccines.** We have obtained crystals of the covNHR antigen in two different crystallization conditions. These small protein constructs are derived from the gp41 HIV-1 protein and have been rationally engineered to display well known neutralizing epitopes of gp41, as well as being stable, soluble and easily producible by E. coli expression in recombinant form. Previously, we collected data at 2.5 Å from crystals grown in ammonium sulphate in the beamline XALOC of the ALBA synchrotron (Barcelona, Spain). We have a molecular replacement solution and at this beamtime allocation we wanted to improve the data resolution.

| Table 1.- Crystals samples from the UAL laboratory. | | | | |
|---|---|---|---|---|
| **Xtal (Protein)** | **# samples** | **Xtal Conditions** | **Diffraction** | **Data collected** |
| COV NHR ABC | 10 | pH 4-8 0.1M buffer NaCl/$(NH_4)_2SO_4$; Cryo Mitigen oil | No | No |
| Tandem W1W2 domain from YAP65 | 10 | PEG 8k/$(NH_4)_2SO_4$ | No | No |
| WW2 domain from YAP65 | 10 | PEG 8k/$(NH_4)_2SO_4$ | No | No |
| WW1 domain from YAP65 | 10 | pH5, 0.1 M buffer, $(NH_4)_2SO_4$,5mM + additives | No | No |
| c-Src-SH3 domain/NS5A complex | 3 | pH4 0.1M AcONa, $(NH_4)_2SO_4$ | No | No |
| Alpha spectrin-SH3 domain | 7 | pH3-8, 0.1 M buffer, $(NH_4)_2SO_4$ | No | No |

Crystal from the dewar coming from Granada by project:

**i) Diffraction studies of ancestral thioredoxin.** We have already crystallized several of those anciant TRXs and the structures hasve been solved from ESRF data collection and deposited at the PDB (2YJ7, 2YNX, 2YPM, 2YN1, 2YOI, 3ZIV and 4BA7) [1-2]. We intended to improve the difraction resolution of the AECA deposited at a resolution of 2.65 Å (3ziv.db and report of the 1st year Mx1406) and to obtain the structure of the K90L mutant of the LPBCA ancestral thioredoxin. We have solved the structure of LPBCA-L89K mutant by molecular replecement using the structure of LPBCA (2YJ7.pdb) as initial model (Table 3) [3].

1. Perez-Jimenez, R., Ingles-Prieto, A., Zhao, Z.M., Sanchez-Romero, I., Alegre-Cebollada, J., Kosuri, P., Garcia-Manyes, S., Kappock, T.J., Tanokura, M., Holmgren, A., et al. (2011). Single-molecule paleoenzymology probes the chemistry of resurrected enzymes. Nature structural & molecular biology 18, 592-596.
2. A. Ingles-Prieto, B. Ibarra-Molero, A. Delgado-Delgado, R. Perez-Jimenez, J. M. Fernandez, E. A. Gaucher, J. M. Sanchez-Ruiz and J. A. Gavira. "Conservation of Protein Structure over Four Billion Years." *Structure*, (2013), doi: 10.1016/j.str.2013.06.020.
3. Risso, V. A., Manssour-Triedo, F., Delgado-Delgado, A., Arco, R., Barroso-delJesus, A., Ingles-Prieto, A., Godoy-Ruiz, R., Gavira, J. A., Gaucher, E. A., Ibarra-Molero, B. and Sanchez-Ruiz, J. M. "Mutational Studies on Resurrected Ancestral Proteins Reveal Conservation of Site-Specific Amino Acid Preferences throughout Evolutionary History" Mol Biol Evol, (2015), doi: 10.1093/molbev/msu312.

**ii) Structural determination of Pseudomonas chemotactic transducer A and B.** To elucidate the way of binding of these co-factors, we have produced crystals of PctA and PctB pre-incubated with several of their natural ligands. Preliminary results form ID14-4 (ESRF) have already been pubished [3] but improved diffraction quality for other ligands are still undergoing.

3. M. Rico-Jiménez, F. Muñoz-Martínez, T. Krell1, J. A. Gavira and E Pineda-Molina. "Purification, crystallization and preliminary crystallographic analysis of the ligand binding regions of the PctA and PctB chemoreceptors from Pseudomonas aeruginosa in complex with amino acids." *Acta Crystallographica, F69 1431-1435*, doi:10.1107/S1744309113023592.

**iii) LBD-McpS bounded to several ligands.** McpS is a chemoreceptor able to recognize specifically 6 of the 7 intermediates of the TCA cycle. We have solved the structure of the McpS-LBD together with two of its main co-factors, malic acid and succinate, at 1.8 and 1.9 Å respectively (PDB ID. 2YFA and 2YFB) [4-5] and perused the crystallization of the LBD-McpS bounded to citrate, benzoate, etc.

4. Evidence for chemoreceptors with bimodular ligand binding region harboring two signal-binding sites. *PNAS,* (2012). 109, 18926-31. Pineda-Molina, E.; Lacal, J.; Reyes-Darias, J. A.; Ramos, J. L.; J. M. Garcia-Ruiz; Gavira, J. A.; Krell, T. doi: 10.1073/pnas.1201400109.
5. Crystallization and crystallographic analysis of the ligand-binding domain of the Pseudomonas putida chemoreceptor McpS in complex with malate and succinate. *Acta. Cryst.,* (2012). F68, 428-431. J. A. Gavira, J. Lacal, J. L. Ramos, J. M. García-Ruiz, T. Krell and E. Pineda-Molina. doi: 10.1107/S1744309112004940.

**iv) Radiation damage studies.** We have also included several samples of lysozyme and glucose isomerase of a starting collaborative project between the Almeria and Granada group as diffraction quality initial test.

| Table 2.- Crystals samples from Granada dewar. | | | | |
|---|---|---|---|---|
| **Protein** | **# Samples** | **Conditions** | **Cryo/s** | **Results** |
| LPBCA L89K | 9 | 0.1M Na Acetate pH 4.0, 20% PEG 400, 15% PEG 4000 & 10% PEG 8000 | No cryo | 2 full data sets. Both of them at a resolution below 2.4 Å and with anisotropic problems. |
| Trx324 (AECA) | 1 | 25% PEG 4000, 0.2M AS & 100mM Na Acetate pH 4.6 | 15% Glycerol | Poor diffraction. |
| PctA_1 | 4 | 3M AS & 0.1M Na Acetate pH 4.0 | 20% Glycerol/ No cryo | Bad diffraction & detector problem. |
| PctA_2 | 6 | 3M AS & 0.1M Na Acetate pH 5.0 | 20% Glycerol/ No cryo | Detector problem |
| McpS - Cit | 10 | 0.1M Mes pH 5.8, 20% PEG 4000 & 0.15M AS | 20% PEG 400 | Poor or no diffraction. |
| McpS - Bzn | 10 | 0.1M Mes pH 5.0, 20% PEG 4000 & 0.25M AS | 20% PEG 400 | Poor diffraction. One date set at 10 Å. |
| Lisozima | 8 | Agarose/Gel 2.6 | No cryo | 2 full data sets |

| | | | | |
|---|---|---|---|---|
| | | | 20% Glycerol | 2 full data sets |
| Glucy | 2 | Agarose/Gel 2.6 | No cryo | Ice rings |
| | | | 20% Glycerol | 1 Full data set |

Table 3. Data collection and refinement statistics (values in parentheses are for the highest-resolution shell).

| | |
|---|---|
| | LPBCA L89K |
| **PDB identifier** | **4ulx** |
| **Data collection** | |
| Beam line | BM30A (ESRF) |
| Space Group | *P 212121* |
| Cell dimensions | |
| a, b, c (Å) | 35.17, 38.88, 61.04 |
| ASU | 1 |
| Resolution (Å) * | 32.79 - 2.35 (2.434 - 2.35) |
| $R_{merge}$ * | 0.1701 (1.069) |
| $I/\sigma_I$ * | 11.14 (2.06) |
| Completeness (%)* | 99.95 (100.00) |
| Unique reflections* | 3761 (366) |
| Multiplicity* | 6.8 (7.0) |
| CC(1/2) | 0.996 (0.707) |
| **Refinement** | |
| Resolution (Å) | 32.79 - 2.35 |
| $R_{work}/R_{free}$ (%) | 19.92 / 24.37 |
| No. atoms | 894 |
| Protein | 877 |
| Ligands | 1 |
| Water | 16 |
| Average B-factors (Å$^2$) | 48.4 |
| R.m.s deviations | |
| Bond lengths (Å) | 0.003 |
| Bond angles ($^0$) | 0.97 |
| Ramachandran (%) | |
| Favored | 97 |
| Outliers | 0 |