ESRF	**Experiment title: Macromolecular Crystallography at South-East Andalusia**	**Experiment number**: MX-1739
Beamline: ID30A-1	**Date of experiment**: From: 17/06/2015 to: 17/06/2015	**Date of report**: 02/08/16
Shifts: 1	**Local contact(s)**: Didier NURIZZO (didier.nurizzo@esrf.fr)	*Received at ESRF:*

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This is up-dated report of the data collected at ID30A-1 during the second round of MX-1739. We send to the ESRF 50 samples from the teams CSIC and UGR. All sample were tested and those indexed were collected.

Crystals from CSIC-UGR (Granada):

Ancestral Lactamases vs consensus reconstruction.

Delving on the possible evolutionary track of Gram-positive bacteria lactamases. Laboratory resurrection of ancestral proteins allows exploring aspects of ancient life that cannot be inferred from fossil records alone. Experimental resurrection of ancestral proteins also provides access to previously unexplored regions of the properties/sequence space. Due to the inherent difficulties of the ARS methodology, further evaluation of this methodology is being carried out to ensure its biotechnological potential. After previous successful studies on the resurrection of ancestral lactamases belonging to the Gram-negative evolutionary track carried out by our group [1-3], we embarked in the resurrection and characterization of the evolutionary track of Gram-positive bacteria lactamases, to further ensure the applicability of this technique as a standard technique for Protein Engineering laboratories.

Related to the studies on ancestral enzyme reconstruction, we have developed and evaluated different reconstruction methodologies -more simple than ARS- using the natural sequence diversity available at different sequence databases. Among other properties, we have specifically investigated the propensity of different reconstructed enzymes to crystallize. An in-depth crystallization study of computationally-engineered beta-lactamases has been carried out, showing that the evolutionary information provided by natural sequence diversity can be exploited as a new methodology for the improvement of protein crystallizability. In this sense, we have measured 47 crystals corresponding to different contruct and crystallization conditions. We have produce 22 useful data set at resolution in the range of the 2.0 Å and therefore we may produced 22 new 3D models. There are also several polymorhp depending on the construct and the crystallization conditions. These results are expected to be published in the very near future.

Future perpectives: This line of investigation should end at this point with the publication of the data obtained to date and the deposit of the 3D structural models.

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2. Valeria A. Risso, Jose A. Gavira, Diego F. Mejia-Carmona, Eric A. Gaucher, and Jose M. Sanchez-Ruiz, Hyperstability and Substrate Promiscuity in Laboratory Resurrections of Precambrian β-Lactamases. JACs **135** (2013) 2899-902

3. Zou, T., Risso, V. A., Gavira, J. A., Sanchez-Ruiz, J. M. and Ozkan, S. B. *Evolution of conformational dynamics determines the conversion of a promiscuous generalist into a specialist enzyme.* Mol Biol Evol, **32** (2015) 132-43.