CRYSTALLOGRAPHY AND PROTEIN ENGINEERING UNIT

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OVERVIEW

Nowadays, knowledge of the three-dimensional (3D) structure of a protein is critical in order to gain a full understanding of its function. The structures of proteins, alone or in complex with other biological partners, reveal functional networks thereby providing a better understanding of the behaviour of the cell's molecular machinery. This implies knowing how proteins move, detecting their interacting partners, and comprehending the changes that they undergo. Close images facilitated by 3D structures provide the possibility of introducing rationally designed mutants that alter their affinity and specificity towards interacting molecules, aiding in the recognition of the physicochemical mechanisms that govern their function. This is why structural data has become crucial in guiding the drug design process, and the results have proven to be relevant for the development of novel therapies.

To achieve this goal, the Crystallography and Protein Engineering Unit provides services at different levels in order to meet the demands of research groups at the CNIO and outside our institute. At the structural determination level, the Unit offers state-of-the-art, high-throughput protein crystallisation screening facilities that include sophisticated equipment for the identification of protein crystals, as well as a full-service offering of X-ray crystallography and small-angle x-ray (SAXS) analyses. As an academic Unit, we have access to high-tech European infrastructures such as the synchrotron light sources. At the protein production level, we have at our disposal a wide array of instrumentation and technical support for the design and purification of soluble recombinant proteins required in large amounts up to milligram quantities, for structural, biophysical or biochemical characterisation, and also for antibody production.

RESEARCH HIGHLIGHTS

This has been a year of growth and exciting changes for this 'new' Crystallography and Protein Engineering Unit. It is the result of the inclusion of CNIO's protein production facility, previously integrated in the Proteomics Unit (Biotechnology Programme), in the Crystallography Unit. The Unit continues to be shared between the Structural Biology and Biocomputing Programme and the Experimental Therapeutics Programme.

Throughout 2016, we have worked closely with the Experimental Therapeutics Programme on several projects, some of them also in collaboration with other CNIO Groups. The scaling up of the production of proteins, like full-length human MASTL, has permitted a wide range of biochemical experiments to take place. Other projects were directly focused on structural characterisation by x-ray crystallography in support of drug discovery, as in the case of the human proteins HASPIN and CDK8/CyclinC complex where we obtained several crystal structures of the protein-ligand complexes (FIGURE). Especially relevant was our continuous work on the production of proteins for the generation of antibodies by the CNIO Monoclonal Antibody Unit (Biotechnology Programme). During 2016, this smooth collaboration has led to the production of several proteins involved in cancer such as CDC25A, IDO1, TDO2, IL11, PDL1, PDL2 or NOMO1.

The Unit also undertakes several collaborations with different CNIO groups. It is noteworthy to mention the collaborations established with CNIO's Telomeres and Telomerase Group, the Gastrointestinal Cancer Clinical Research Unit, the Epithelial Carcinogenesis Group and the Structural Computational Biology Group. Additionally, the Unit maintains external collaborations with groups at the Physical Chemistry Department (University of Granada), the Environmental Biology Department (CIB-CSIC), the Pharmacology and Therapeutics Department (Roswell Park Cancer Institute, USA), the Department of Biomedicine (University of Bergen, Norway), and the Department of Molecular Engineering (Åarhus University, Denmark).

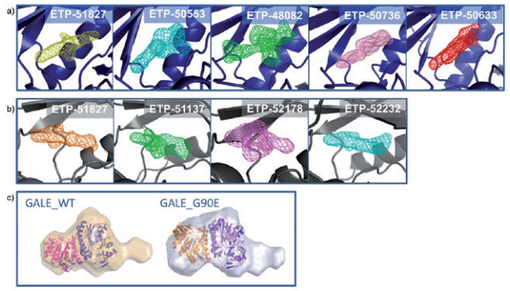

Figure Close views of the active sites from the x-ray structures of HASPIN (**A**) and CDK8/CyclinC (**B**). The coloured omit maps correspond to the electron density of the bound compounds (synthesised in CNIO's Medicinal Chemistry Section). (**C**) SAXS *ab initio* shape reconstructions of wild type UDP-galactose 4'-epimerase (GALE) and its mutant G90E, superimposed on the crystal structure. The data explains the drastic conformational changes that reduce NAD+ binding affinity in the mutant, causing type III galactosemia. This work was done in collaboration with the Physical Chemistry Department (University of Granada, Spain).

Finally, the Unit has continued the study of the role of ephrinB2 in different pathologies. This was done by blocking its activity with specific recombinant antibodies generated by us, in collaboration with groups from the MRC Clinical Sciences Centre (UK) and the NCI Center for Cancer Research (USA). ■

PUBLICATIONS

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