CRYSTALLOGRAPHY AND PROTEIN ENGINEERING UNIT

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Unit Head

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OVERVIEW

The Crystallography and Protein Engineering Unit (XTPEUnit) is a core facility created to provide on-demand services at different levels to fulfil the needs of our users. By offering services ranging from protein cloning to solving the 3D structures of proteins, we help our users to further comprehend how target proteins work. With this purpose in mind, we produce proteins for different types of biochemical/biophysical/*in vitro*/*in vivo* assays and for monoclonal antibody production, also offering macromolecular structural determination at high-resolution (atomic) by X-ray crystallography, and at low-resolution in solution by small-angle X-ray scattering (SAXS). Protein co-crystallisation, in the presence of inhibitors or small fragments, is routinely done in our laboratory in combination with studies on protein thermal stability (thermofluor assay), to accelerate the guided drug discovery process.

"By fragment screening on crystals, we visualise direct interactions between small molecules and proteins, speeding up the identification of new targetable sites in drug discovery projects."

Postdoctoral Fellows
Raquel S. Correia (until March)·
Johanne Le Coq (until December),
Virginia Sandonís (since October)

Technicians
Aida Contreras (TS) * (PEJ) **, Diana Martín (since February), Álvaro Otero (TS) *, Eduardo Rebollo (TS) * (PEJ) **, Pilar Redondo (since December)

**Plan de Empleo Joven* (Youth Employment Plan)
***Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

Our Unit works closely with the Experimental Therapeutics Programme on several projects. To fulfil the need of recombinant proteins, we produced, throughout the year, full-length and kinase domain human MASTL, full-length mouse TRF1 and human TRF1 dimerization domain, for biochemical, *in vitro*, thermo-stability and structural analyses. Furthermore, to support drug discovery projects, we performed several thermal shift assays (thermofluor) in the presence of compounds developed and tested at the Medicinal Chemistry Section and the Biology Section, respectively.

We also continued our close collaboration with the CNIO Monoclonal Antibodies Unit on the production of proteins to generate highly specific antibodies against several cancer-associated proteins such as HASPIN, RANK, CD85C, CD85G and CD85J, and other protein tools such as Cas9. Additionally, we ran a number of internal collaborations with other CNIO Groups and Units, providing them with recombinant proteins for biochemical and/or cell-based functional assays; this was the case, for example, with the Telomeres and Telomerase Group, the Experimental Oncology Group, the Genomic Instability Group, the Cell Division and Cancer Group, the Melanoma Group, the H12O-CNIO Lung Cancer Clinical Research Unit, the Macromolecular Complexes in DNA Damage Response Group, the H12O-CNIO Haematological Malignancies Clinical Research Unit, and the Transformation and Metastasis Group.

The Unit maintained collaborations with various external groups: the Environmental Biology Department, *CIB-CSIC*, Spain; the Pharmacology and Therapeutics Department, Roswell Park Cancer Institute, USA; the Department of Biomedicine, University of Bergen, Norway; the Department of Crystallography and Structural Biology, *Instituto Quimica-Fisica Rocasolano*, *CSIC*, Spain; the Department of Immunology, Genetics and Pathology, Uppsala University, Sweden; the Cancer Immunotherapy Unit; *12 de Octubre* University Hospital, Spain; and the Division of Pulmonary and Critical Care Medicine, Fibrosis Research Center, and Center for Immunology and Inflammatory Diseases, Harvard Medical School, USA.

Throughout 2020, the Unit also proceeded with its own scientific projects. We continued working on targeting the function of the Mdm2-MdmX E3 complex activity in the context of an NIH-funded collaborative project with the Department of Pharmacology and Therapeutics at Roswell Park Cancer Institute. In addition, we are recombinantly producing a T cell-recruiting bispecific antibody (named *ATTACK*) for structural and functional purposes, in collaboration with the company LeadArtis, the Department of Microbiology (Immunology) of the *Complutense* University of Madrid, and the Cancer Immunotherapy Unit of the *12 de Octubre* University Hospital; a project funded by the *Retos Colaboración* programme of the Spanish Ministry of Science, Innovation and Universities. The Unit is also taking part in 2 collaborative projects with the Biomedical Application of Radioisotopes Unit of *CIEMAT*, the Bioactive Nanostructured Materials Group of the *Complutense* University of Madrid, and the CNIO's Molecular Imaging Unit to develop new antibody-based positron emission tomography (immunoPET) imaging tools for tumour visualisation and pretargeted clickable antibody fragments for theranostic applications; both projects are supported by *BBVA* Foundation grants. Finally, in 2020 we were awarded a *BBVA* Foundation grant, jointly with the Cancer Immunotherapy Unit of the *12 de Octubre* Hospital's Research Institute (i+12), to design a new immunotherapy method to fight Covid-19. ■

PUBLICATIONS

- Esteban-Burgos L, Wang H, Nieto P, Zheng J, Blanco-Aparicio C, Varela C, Gómez-López G, Fernández-García F, Sanclemente M, Guerra C, Drosten M, Galán J, Caleiras E, Martínez-Torrecuadrada J, Fajas L, Peng SB, Santamaría D, Musteanu M, Barbacid M (2020). Tumor regression and resistance mechanisms upon CDK4 and RAF1 inactivation in KRAS/P53 mutant lung adenocarcinomas. *Proc Natl Acad Sci USA* 117, 24415-24426.
- Frye M, Stritt S, Ortsäter H, Hernandez Vasquez M, Kaakinen M, Vicente A, Wiseman J, Eklund L, Martínez-Torrecuadrada JL, Vestweber D, Mäkinen T (2020). EphrinB2-EphB4 signalling provides Rho-mediated homeostatic control of lymphatic endothelial cell junction integrity. *Elife* 9, e57732.
- Martínez-González S, García AB, Albarrán MI, Cebriá A, Amezquita-Alves A, García-Campos FJ, Martínez-Gago J, Martínez-Torrecuadrada J, Muñoz I, Blanco-Aparicio C, Pastor J (2020). Pyrido[2,3-b][1,5]benzoxazepin-5(6H)-one derivatives as CDK8 inhibitors. *Eur J Med Chem* 201, 112443.

PATENT

- Paz-Ares L, Martinez JL, Roncador G, Ojeda L, Ferrer I (2020). Interleukin 11 receptor alpha subunit (IL11RA) neutralizing antibodies and uses thereof. *EP20382631.8*