CRYSTALLOGRAPHY AND PROTEIN ENGINEERING UNIT

Inés Muñoz
Head of Unit

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OVERVIEW

The Crystallography and Protein Engineering Unit (XTPEUnit) is a central core facility as well as a research laboratory whose main goal is to supply the requests of the research groups within the CNIO, as well as external groups, by providing on-demand services at different levels. The Unit covers all the services that range from the coding sequence cloning to the resolution of the 3D structure, whilst always focusing on helping the research groups in reaching a broader characterisation of their proteins of interest. These services span the production of proteins for different types of biochemical/biophysical assays, monoclonal antibody production, and macromolecular structural determination at low resolution by small-angle X-ray scattering (SAXS) or at high resolution by X-ray crystallography. The latter includes protein co-crystallisation – a method that we routinely combine with the quantification of the protein thermal stability (thermofluor assay) – in the presence of inhibitors during the structure guided drug discovery process.

"We aim to show how protein information at atomic resolution is crucial for understanding the processes occurring in the biology of cancer."

Graduate Student
Silvia L. Gomes (until January)

Technicians
Daniel Calvo (until January) (PEJ, CAM) *(TS) **, Leticia Martín (June-December), Miguel A. Navarro (PEJ, CAM) *, Álvaro Otero (TS) **, Federico Ruiz (TS) **, Alicia Virseda (until April) (TS) **

*_Plan de Empleo Joven de la Comunidad de Madrid_ (Youth Employment Plan, Community of Madrid)
**_Titulado Superior_ (Advanced Degree)

RESEARCH HIGHLIGHTS

The Unit works closely with the Experimental Therapeutics Programme on several projects that have since led to the production of recombinant proteins (full-length human MASTL, full-length mouse TRF1 and human TRF1 dimerization domain) for biochemical experiments. We also focus on structural characterisation by X-ray crystallography in support of drug discovery projects, as in the case of the kinase HASPIN – crystallised in the presence of compounds developed at the Medicinal Chemistry Section (FIGURE) – and other projects like MASTL, DDR1 and the dimerization domain of TRF1 (FIGURE), the latter in strict collaboration with the Telomeres and Telomerase Group. We have continued our work on the production of proteins for the generation of antibodies by the Monoclonal Antibody Unit (Biotechnology Programme), including several cancer-involved proteins such as IL11R, TET2, NGFR, ECT2, CDK16, Pot1A, MIDKINE, GPSM3, Ly9, CD85A, and HASPIN, among others. The Unit is also engaged in numerous internal collaborations with other CNIO Research Groups and Units; noteworthy are the following: Telomeres and Telomerase, Experimental Oncology, Genomic Instability, Cell Division and Cancer, Molecular Imaging Unit, Microenvironment and Metastasis, Gastrointestinal Cancer Clinical Research Unit, Melanoma, Epithelial Carcinogenesis, Familial Cancer Unit, H12O-CNIO Lung Cancer Clinical Research Unit, and the H12O-CNIO Haematological Malignancies Clinical Research Unit. Additionally, our Unit has ongoing collaborations with external groups such as the Environmental Biology Department (*CIB-CSIC*), the Pharmacology and Therapeutics Department (Roswell Park Cancer Institute, USA), the Protein Tools Unit (*CNB-CSIC*), the Biomedical Application of Radioisotopes Unit (*CIEMAT*), the Department of Molecular Engineering (Aarhus University, Denmark) and the IVI Foundation.

Throughout 2018, the Unit also sustained its own scientific projects. We continued working on targeting the function of the Mdm2-MdmX E3 complex activity, in collaboration with the Department of Pharmacology and Therapeutics at Roswell Park Cancer Institute. Also, we have solved, by SAXS, the structure in-solution of two mAbs that correspond to the first generation 4-1BB agonistic IgG 1D8, and which are specific tumour-targeted trimerbodies (FIGURE). Our collaborators, at the Department of Molecular Engineering (Denmark), have shown their high tumour inhibition potency *in vivo*. Finally, the Unit is taking part in a project, in collaboration with the Biomedical Application of Radioisotopes Unit at CIEMAT and the CNIO's Molecular Imaging Unit, to develop new antibody-based positron emission tomography (immunoPET) imaging tools for tumour visualisation. ■

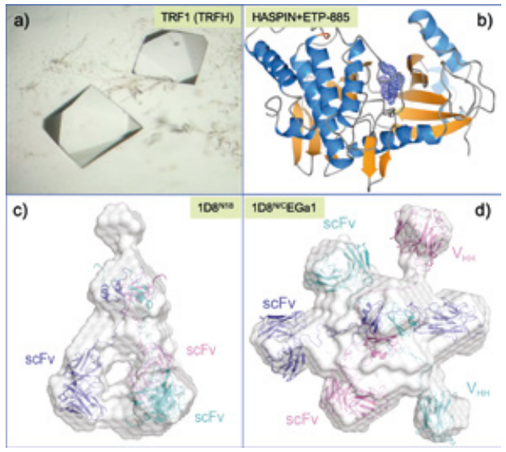

Figure (**a**) Protein crystals of TRF1 dimerization domain. (**b**) X-ray structure of HASPIN in complex with the drug ETP-885. (**c,d**) SAXS study of two trimerbodies developed for cancer therapy. Fit of the *ab initio* SAXS structures (envelopes in grey) and the corresponding generated trimerbodies models (chains in blue, magenta and cyan).

PUBLICATIONS

- Compte M *et al.* (incl. Muñoz I, Martínez-Torrecuadrada J) (2018). A tumor-targeted trimeric 4-1BB-agonistic antibody induces potent anti-tumor immunity without systemic toxicity. *Nat Commun* 9, 4809.
- Mortuza GB *et al.* (incl. Muñoz I) (2018). Molecular basis of Tousled-Like Kinase 2 activation. *Nat Commun* 9, 2535.
- Toledo RA *et al.* (incl. Otero A, Martínez-Torrecuadrada J) (2018). Exome sequencing of plasma DNA portrays the mutation landscape of colorectal cancer and discovers mutated VEGFR2 receptors as modulators of anti-angiogenic therapies. *Clin Cancer Res* 24, 3550-3559.
- Morcillo MÁ *et al.* (incl. Martínez-Torrecuadrada J) (2018). MT1-MMP as a PET imaging biomarker for pancreas cancer management. *Contrast Media Mol Imaging* 2018, 8382148.

AWARDS AND RECOGNITION

- Member of the Board of Directors, *Asociación de Usuarios de Sincrotrón de España.*