CRYSTALLOGRAPHY AND PROTEIN ENGINEERING UNIT

Inés Muñoz
Unit Head

Staff Scientist
Jorge L. Martínez

Postdoctoral Fellow
Yudhi Nugraha (since March)

Technicians
Aida Contreras (TS) * (PEJ) **,
Diana Martín (until April),
Álvaro Otero (until February) (PTA)***
(TS)*, Lluvia Rebollo (TS)* (PEJ)**,
Pilar Redondo, César Rodríguez
(February-September)

* *Titulado Superior* (Advanced Degree)
** *Plan de Empleo Joven* (Youth Employment Plan)
*** *Personal Técnico de Apoyo* (Technical Support Staff, MCI)

OVERVIEW

The Crystallography and Protein Engineering Unit is a core facility that provides on-demand services at different levels, from the cloning, expression and purification of proteins to the determination of their 3D structures, with the purpose to fulfil the demands of our users and to understand the function of their protein targets. Thus, we produce proteins for different types of biochemical/biophysical/*in vitro*/*in vivo* assays, antibody generation, and structural determination at low resolution by small-angle X-ray scattering (SAXS) or at atomic resolution by X-ray crystallography. The latter includes protein co-crystallisation in the presence of inhibitors or small fragments, a method that we routinely combine with the quantification of protein thermal stability (thermofluor assay) to aid the drug discovery process.

"Fragment screening on crystals is regularly used to map the interactions of these small molecules with proteins, speeding up drug discovery projects, not only in industry but also in academic groups."

RESEARCH HIGHLIGHTS

The goal of fragment-based screening is to expose protein crystals to libraries of fragments and to solve the crystal structures of the complexes. Our first target was the dimerization domain of TRF1 (Telomeres and Telomerase Group). We identified 3 well-defined fragments bound to the protein that could be further exploited to develop new inhibitors.

Our Unit works closely with the Experimental Therapeutics Programme on several projects: human TRF1 dimerization domain, the kinase domains of human MASTL and human DDR1 for biochemical and structural analyses. Furthermore, to support drug discovery projects, we performed several thermal shift assays (thermofluor) in the presence of compounds developed in the Medicinal Chemistry Section.

We provide the proteins needed by the CNIO Monoclonal Antibodies Unit to generate antibodies, including the CD85 family, CSF3R, CLLU1, RANK, TACI and PILRA, among others. The Unit is also engaged in several internal collaborations with other CNIO groups, providing them with recombinant proteins for biochemical and/or cell-based functional assays.

Throughout 2021, the Unit also sustained its own scientific projects. We continued working on targeting the function of the Mdm2-MdmX E3 complex activity (NIH funded) as well as on the production of a T cell-recruiting bispecific antibody, ATTACK (funded by *Retos Colaboración*). The Unit is also taking part in 2 projects supported by BBVA Foundation grants. The first is a collaborative project 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 visualization. The second project, carried out in collaboration with the Immuno-oncology and Immunotherapy Unit at the *Hospital 12 de Octubre*, has resulted in the generation of 2 new synthetic nanobodies capable of targeting the spike protein of the SARS-CoV-2 virus. These nanobodies will soon be tested in mouse models at the Poxvirus and Vaccine Laboratory by M. Esteban's group at the *CNB*. ■

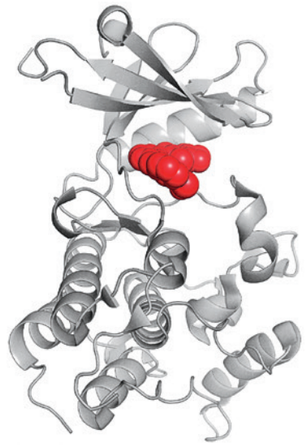

FIGURE Three-dimensional crystal structure of the DDR1 kinase domain (in grey) in complex with the drug ETP-078 (in red).

PUBLICATIONS

- Sanclemente M, Nieto P, García-Alonso S, Fernández-García F, Esteban-Burgos L, Guerra C, Drosten M, Caleiras E, Martinez-Torrecuadrada J, Santamaría D, Musteanu M, Barbacid M (2021). RAF1 kinase activity is dispensable for KRAS/p53 mutant lung tumor progression. *Cancer Cell* 39, 294-296.
- Compte M, Harwood SL, Erce-Llamazares A, Tapia-Galisteo A, Romero E, Ferrer I, Garrido-Martin EM, Enguita AB, Ochoa MC, Blanco B, Oteo M, Merino N, Nehme-Álvarez D, Hangiu O, Domínguez-Alonso C, Zonca M, Ramírez-Fernández A, Blanco FJ, Morcillo MA, Muñoz IG, Melero I, Rodriguez-Peralto JL, Paz-Ares L, Sanz L, Alvarez-Vallina L (2021). An Fc-free EGFR-specific 4-1BB-agonistic trimerbody displays broad antitumor activity in humanized murine cancer models without toxicity. *Clin Cancer Res* 27, 3167-3177.
- Compte M, Harwood SL, Martínez-Torrecuadrada J, Perez-Chacon G, González-García P, Tapia-Galisteo A, Van Bergen En Henegouwen PMP, Sánchez A, Fabregat I, Sanz L, Zapata JM, Alvarez-Vallina L (2021). Case report: an EGFR-targeted 4-1BB-agonistic trimerbody does not induce hepatotoxicity in transgenic mice with liver expression of human EGFR. *Front Immunol* 11, 614363.
- Martínez-Caballero S, Mahasenan KV, Kim C, Molina R, Feltzer R, Lee M, Bouley R, Hesek D, Fisher JF, Muñoz IG, Chang M, Mobashery S, Hermoso JA (2021). Integrative structural biology of the penicillin-binding protein-1 from *Staphylococcus aureus*, an essential component of the divisome machinery. *Comput Struct Biotechnol J* 19, 5392-5405.

PATENT

- Paz-Ares L, Torrecuadrada Martínez JL, Roncador G, Ojeda L, Ferrer I (2020). Interleukin 11 receptor alpha subunit (IL-11RA) neutralizing antibodies and uses thereof. *PCT/EP2021/069630*. PCT application (2021).