PROTEIN CRYSTALLOGRAPHY UNIT

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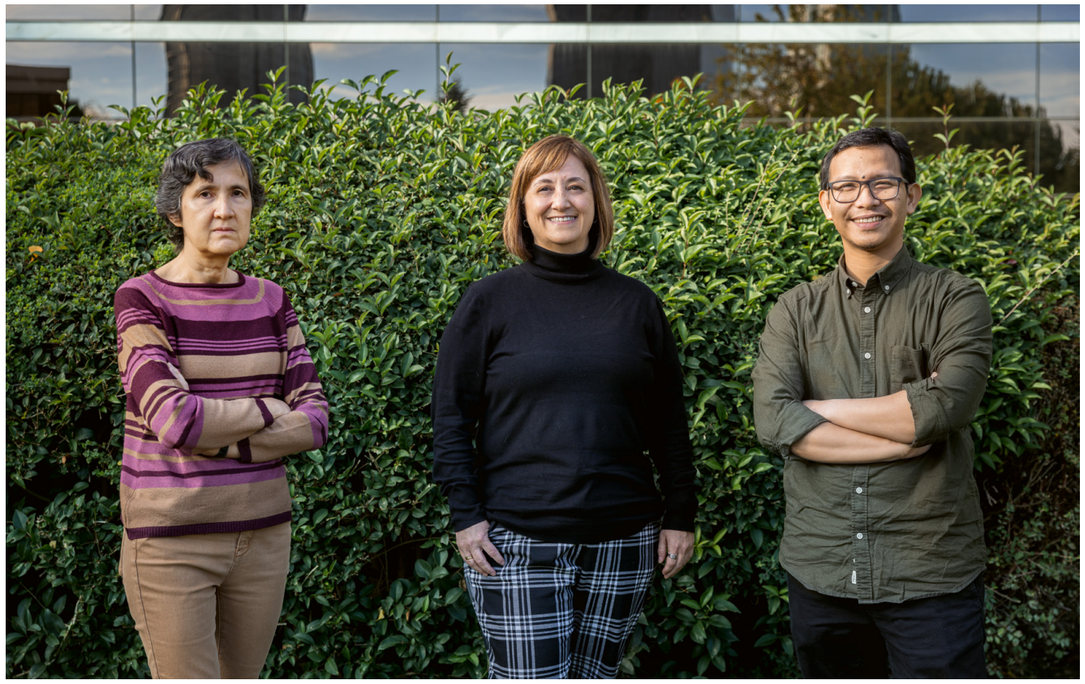

OVERVIEW

The Protein Crystallography 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 high-quality proteins for different types of assays 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 helps to map new binding sites in the target proteins."

RESEARCH HIGHLIGHTS

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

The Unit is also engaged in several internal collaborations with other CNIO groups (Growth Factors, Nutrients and Cancer; Transformation and Metastasis; Metabolism and Cell Signalling; Experimental Oncology; Microenvironment and Metastasis; Topology and DNA Breaks; DNA Replication; Macromolecular Complexes in DNA Damage Response; Kinases, Protein Phosphorylation and Cancer Groups; and the H12O-CNIO Lung Cancer Clinical Research Unit), providing some of them with recombinant proteins that can be used for protein crystallography, SAXS or thermofluor assays analysis and, in some cases, for other biophysical, biochemical, cell-based functional assays and cryoEM studies.

Throughout 2022, the Unit also continued working on its own scientific project, supported by a grant from the BBVA Foundation. Carried out in collaboration with the Immuno-oncology and Immunotherapy Unit at the *Hospital 12 de Octubre*, this work generated a new synthetic bispecific antibody capable of targeting the spike protein of the SARS-CoV-2 virus, inducing neutralisation while promoting T cell cross-priming. We also revealed the cryo-EM structure, which shows how the trimerbody (TN^T) binds the trimeric RBD spike ectodomain in a 1:1 equimolar ratio. ■

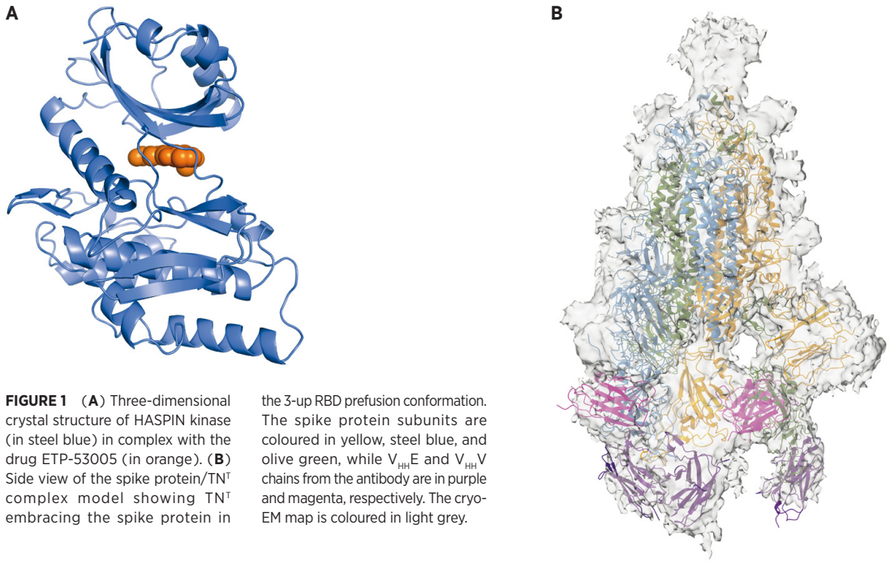

FIGURE 1 (**A**) Three-dimensional crystal structure of HASPIN kinase (in steel blue) in complex with the drug ETP-53005 (in orange). (**B**) Side view of the spike protein/TN^T complex model showing TN^T embracing the spike protein in the 3-up RBD prefusion conformation. The spike protein subunits are coloured in yellow, steel blue, and olive green, while $V_{HH}E$ and $V_{HH}V$ chains from the antibody are in purple and magenta, respectively. The cryo-EM map is coloured in light grey.

PUBLICATION

Lama R, Xu C, Galster SL, Querol-García J, Portwood S, Mavis CK, Ruiz FM, Martin D, Wu J, Giorgi MC, Bargonetti J, Wang ES, Hernandez-Ilizaliturri FJ, Koudelka GB, Chemler SR, Muñoz IG, Wang X (2022). Small molecule MMRi62 targets MDM4 for degradation and induces leukemic cell apoptosis regardless of p53 status. *Front Oncol* 12, 933446.