PROTEIN CRYSTALLOGRAPHY UNIT

Inés Muñoz
Unit Head

Postdoctoral Fellow
Yudhi Nugraha (until March)

Technician
Pilar Redondo (until May)

OVERVIEW

The Protein Crystallography Unit is a core facility that provides on-demand services at different levels, from the cloning, expression, and purification of high-quality 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 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.

"Understanding the three-dimensional crystal structure of proteins, including their behaviour in solution, is fundamental to life science and biopharmaceutical researchers."

Student in Practice
Daffa Adinegoro (until June)
(PhD student, Graduate School of Bioagricultural Sciences, Nagoya University, Japan)

RESEARCH HIGHLIGHTS

Our Unit works closely with the Experimental Therapeutics Programme on several projects in support of drug discovery. This includes the kinase domain of the human protein HASPIN for biochemical and structural analyses in the presence of new 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; Experimental Oncology; Microenvironment and Metastasis; DNA Replication; Macromolecular Complexes in DNA Damage Response; and Kinases, Protein Phosphorylation and Cancer Groups; the H12O-CNIO Lung Cancer Clinical Research Unit; and the H12O-CNIO Cancer Immunotherapy Clinical Research Unit), providing recombinant proteins that were used for protein crystallography, SAXS or thermofluor assay analysis and, in some cases, for other biophysical, biochemical, cell-based functional assays and cryoEM studies.

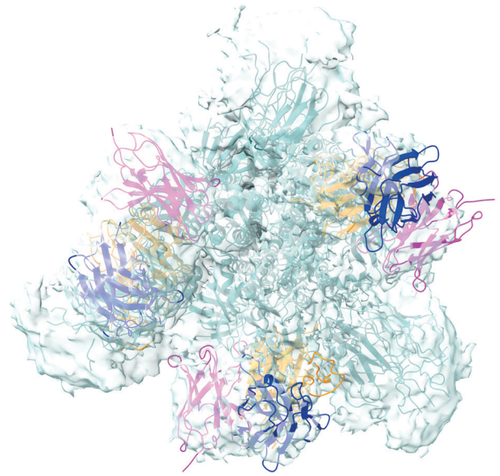

FIGURE 1 Top view of the spike protein/TN^T complex model showing TN^T embracing the spike protein in the 3-up RBD prefusion conformation. To enhance its visualisation, the spike protein was coloured in pale blue while its 3 RBD subunits were coloured in yellow. The $V_{HH}E$ and $V_{HH}V$ chains from the synthetic antibody are in purple and magenta, respectively. The cryoEM map is painted in light grey. The figure shows how each pair of antibody chains embraces one RBD, thus structurally demonstrating their neutralising effect.

Throughout 2023, the Unit also continued working on its own scientific project, supported by a grant from the *BBVA* Foundation. Carried out in collaboration with the H12O-CNIO Cancer Immunotherapy Clinical Research Unit, this research 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 were involved in solving the cryoEM structure, which shows how the trimerbody (TNT) binds the trimeric RBD spike ectodomain in a 1:1 equimolar ratio (FIGURE 1). In addition, the Unit maintained collaborations with various external groups in Spain: the Department of Crystallography and Structural Biology (*IQF-CSIC*, Madrid), and the Molecular Mechanisms and Experimental Therapy in Oncology Programme (*IDIBELL*, Barcelona). ■

PUBLICATIONS

- Cuesta-Hernández HN, Contreras J, Soriano-Maldonado P, Sánchez-Wandelmer J, Yeung W, Martín-Hurtado A, Muñoz IG, Kannan N, Llimargas M, Muñoz J, Plaza-Menacho I (2023). An allosteric switch between the activation loop and a c-terminal palindromic phospho-motif controls c-Src function. *Nat Commun* 14, 6548.
- Mur P, Viana-Errasti J, García-Mulero S, Magraner-Pardo L, Muñoz IG, Pons T, Capellá G, Pineda M, Feliubadaló L, Valle L (2023). Recommendations for the classification of germline variants in the exonuclease domain of POLE and POLD1. *Genome Med* 15, 85.
- Martínez-Caballero S, Freton C, Molina R, Bartual SG, Gueguen-Chaignon V, Mercy C, Gago F, Mahasenan KV, Muñoz IG, Lee M, Hesek D, Mobashery S, Hermoso JA, Grangeasse C (2023). Molecular basis of the final step of cell division in *Streptococcus pneumoniae*. *Cell Rep* 42, 112756.
- Lázaro-Gorines R, Pérez P, Heras-Murillo I, Adán-Barrientos I, Albericio G, Astorgano D, Flores S, Luczkowiak J, Labiod N, Harwood SL, Segura-Tudela A, Rubio-Pérez L, Nugraha Y, Shang X, Li Y, Alfonso C, Adipietro KA, Abeyawardhane DL, Navarro R, Compte M, Yu W, MacKerell AD Jr, Sanz L, Weber DJ, Blanco FJ, Esteban M, Pozharski E, Godoy-Ruiz R, Muñoz IG, Delgado R, Sancho D, García-Arriaza J, Álvarez-Vallina L (2023). Dendritic cell-mediated cross-priming by a bispecific neutralizing antibody boosts cytotoxic T cell responses and protects mice against SARS-CoV-2. *Adv Sci* 10, e2304818.

AWARDS AND RECOGNITION

- Inés Muñoz was a member of the scientific panel that made the decision to begin construction, at the ALBA Synchrotron facility (Barcelona, Spain), of 2 new long-beamlines: Cryo bio-nano-imaging beamline (CORUS) and Coherent Diffraction Imaging beamline (CoDI).