

ELECTRON MICROSCOPY UNIT

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OVERVIEW

The principal goal of the Electron Microscopy (EM) Unit is to offer scientific-technical support to researchers to resolve their scientific questions using different transmission EM techniques. We routinely use cryo-EM and negative staining to prepare samples. We also perform data collection and help in image processing, through 2D analysis and 3D reconstruction. Support is offered in choosing adequate EM techniques and performing sample preparation. Moreover, we manufacture our own sample supports (EM grids) for better quality control and lower cost. In addition, we provide the training necessary for the use of our microscopes and auxiliary equipment. More advanced structural studies are generally carried out through research collaboration.

“In the Electron Microscopy Unit we dedicate our central effort to securing efficient access to all our infrastructure. We also offer the training necessary for the use of our microscopes and auxiliary equipment.”

RESEARCH HIGHLIGHTS

In 2022, we gave support to several CNIO Groups in their research activities. In collaboration with the Transformation and Metastasis Group, we analysed mitochondrial morphology in human breast cancer patient-derived xenografts (PDX). Together with the Microenvironment & Metastasis Group, we studied different types of vesicles, and with the Growth Factors, Nutrients and Cancer Group, we optimised cryoEM grids and collected data for structural studies of the URI complex. We also started a collaboration with the H120-CNIO Haematological Malignancies Clinical Research Unit to structurally characterise hnRNPK.

We continued collaborating closely with all the groups from the Structural Biology Programme, performing single-particle EM grid preparation, cryo-EM grid screening, data collection, and 2D and 3D analysis of different samples. We collaborated in several projects carried out by the Macromolecular Complexes in DNA Damage Response Group, performing EM grid preparation, data collection, and analysis of different samples: ARN helicase DDX11; RuvBL complex of *Arabidopsis thaliana* (a collaboration with D. Alabadí, *Universitat Politècnica de València*); lncRNA (a collaboration with M. Huarte, *CIMA, Universidad de Navarra*); and different heteromeric amino acid transporters (a collaboration with M. Palacin and J. Fort, *IRB Barcelona*). With the Genome Integrity and Structural Biology Group, we provided cryoEM grid screening and data collection of different samples, and with the Kinases, Protein Phosphorylation and Cancer Group, we performed EM grid optimisation, data collection and processing of PTC1 Kinase, as well as EM grid preparation and imaging of KIF5B-RET kinesin samples. Outside our Centre, together with Rafael Fernández Leiro, we are collaborating with J.A. Costoya Puente (*Universidad de Santiago de Compostela*) on characterising the structure of human hPARP1. Furthermore, together with E. Lara (*CNIC*), we are studying mitochondrial structure in brown adipose tissue (BAT) of KO CnAbeta1 mice. ■

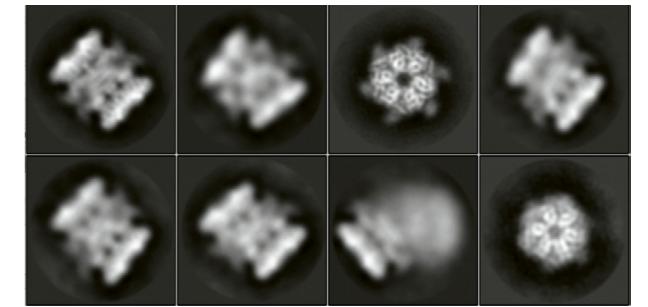


FIGURE 1 Reference-free 2D class averages demonstrating that cryo-electron microscopy is a powerful tool to view the structural flexibility of protein and protein complexes.

• PUBLICATIONS

- Le Coq J, Acebrón I, Rodrigo Martín B, López Navajas P, Lietha D (2022). New insights into FAK structure and function in focal adhesions. *J Cell Sci* 135, jcs259089.
- Rivera-Calzada A, Arribas-Bosacoma R, Ruiz-Ramos A, Escudero-Bravo P, Boskovic J, Fernandez-Leiro R, Oliver AW, Pearl LH, Llorca O (2022). Structural basis for the inactivation of cytosolic DNA sensing by the vaccinia virus. *Nat Commun* 13, 7062.