ELECTRON MICROSCOPY UNIT

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OVERVIEW

The main objective of the Electron Microscopy (EM) Unit is to provide scientific-technical support to researchers to answer their biological questions using different transmission EM techniques. We regularly use negative staining and cryo-EM and help with image processing by performing 2D analysis and 3D reconstruction. We also offer support for choosing adequate EM techniques and performing sample preparation on different types of EM grids. Furthermore, we provide the necessary training for the use of our microscopes and auxiliary equipment. More advanced studies are typically delivered through research collaboration.

"We dedicate our main effort to ensuring efficient access to and use of existing infrastructure in the Unit. We also provide personalised scientific support and training for researchers."

RESEARCH HIGHLIGHTS

In our studies, we take advantage of the continuous technical advances in cryogenic electron microscopy (cryoEM). Specifically, we use single-particle cryoEM to elucidate the structures of macromolecules at near atomic resolution. At the CNIO we have a 120 kV, Tecnai G2 Spirit microscope equipped with a TVIPS CMOS detector that is used to obtain images of negatively stained samples, to screen vitrified samples, and for small-scale data collection. For medium resolution structural studies, we use a JEM-2200FS cryo-electron microscope equipped with a 200 kV field emission gun and a K3 direct electron detector.

Our scientific activity throughout 2021 involved collaborations with the research groups of the Structural Biology Programme, as well as with groups from other Programmes and with scientists outside the CNIO. For example, together with CNIO's Microenvironment and Metastasis Group, we contributed to the analyses of secreted extracellular vesicles (EVs) that influence the tumour microenvironment and promote distal metastasis. In particular, we imaged melanoma-secreted EVs that have been associated with lymph node, pre-metastatic niche formation in murine models. With the Macromolecular Complexes in DNA Damage Response Group, we pursued our work to structurally characterise several protein complexes e.g., different RUVBL1/2 and DNA repair complexes. Our collaboration also continued with M. Palacín's group *(IRB* Barcelona), with whom we contributed to revealing the molecular mechanisms controlling substrate specificity within the heteromeric amino acid transporter (HAT) family of neutral amino acid transporters. These findings provide the structural bases for mutations in LAT2/CD98hc (HAT member) that alter substrate specificity and that are associated with several pathologies. Finally, in collaboration with the Genome Integrity and Structural Biology Group, we further improved our set-up to use a cryoEM as a tool for drug discovery. ■

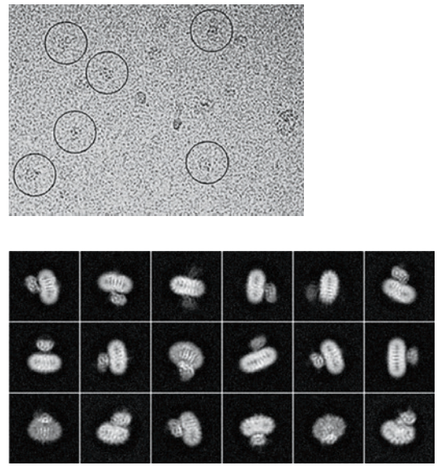

FIGURE CryoEM of the heteromeric amino acid transporter hLAT2/CD98hc embedded in a detergent micelle. (**A**) Representative cryo-electron microscopy field. (**B**) Reference-free 2D class averages.

PUBLICATIONS

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