ELECTRON MICROSCOPY UNIT

Jasminka Boskovic
Unit Head

Post-Doctoral Fellow
Johanne Le Coq (since December)

OVERVIEW

The main goal of the Electron Microscopy (EM) Unit is to provide scientific-technical support to CNIO researchers to solve their biological questions using various transmission EM techniques. We routinely use negative staining and cryo-EM, and we also help in image processing by performing 2D analysis and 3D reconstruction. We also offer support for selecting adequate EM techniques and performing sample preparation. Moreover, we provide the necessary training on the use of our microscopes and auxiliary equipment. More advanced studies are typically delivered through research collaboration.

"We devote our main effort to allowing efficient access to, and use of, existing infrastructure in the Unit. We provide scientific support and training to researchers tailored to their research needs."

Technicians
Carmen García (TS)* (PEJ)**, Pilar Redondo (until December)

*Titulado Superior (Advanced Degree)
**Plan de Empleo Joven (Youth Employment Plan)

Student in Practice
Sofía Sonsoles Doyagüez (February-June) (*Universidad Politéctica de Madrid*, Madid, Spain)

RESEARCH HIGHLIGHTS

Technical advances in the last decade have positioned cryogenic electron microscopy (cryo-EM) as one of the most powerful and effective technologies available to investigate the structures of macromolecules at near-atomic resolution. Among several cryo-EM structural determination methods, single-particle analysis is the most popular for structural biologists, as it has relatively well-established methods for sample preparation, data collection, image processing, and structural determination. At the CNIO we have in place a 120 kV, Tecnai G2 Spirit microscope equipped with a TVIPS CMOS detector that is used to obtain images of negatively stained samples and to screen vitrified samples. For medium resolution structural studies, the Unit is equipped with a JEM-2200FS cryo-EM and a K3 direct electron detector camera. Our scientific activity throughout 2020 involved collaborations with all the Research Groups from the Structural Biology Programme, several Groups from other Programmes, as well as with scientists outside the CNIO. For instance, in collaboration with the Cell Division and Cancer Group, we monitored centriole structure and organisation as a consequence of lack of Cep135, a protein involved in centrosomal and spindle dynamics; in collaboration with the Microenvironment and Metastasis Group, we studied the morphological changes of extracellular vesicles, mainly exosomes isolated from prostate cancer cells; in collaboration with the Macromolecular Complexes in DNA Damage Response Group, we continued our collaboration on the structural characterisation of several protein complexes, e.g., RUVBL1/2 complexes and DNA repair complexes; together with Manuel Palacín's group (*IRB*, Barcelona), we also continued the collaboration on high-resolution structural characterisation of amino acid transporter complexes; and, finally, in collaboration with Genome Integrity and Structural Biology Group, we have been setting-up a pipeline to use a cryo-EM as a tool for drug discovery. ■

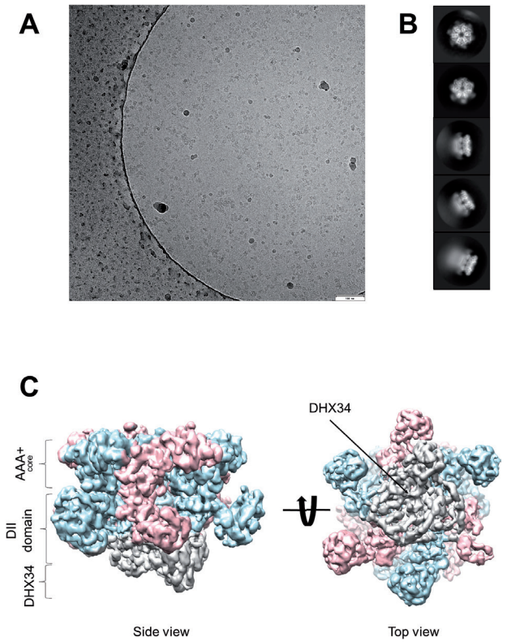

FIGURE Architecture of RUVBL1-2-DHX34 complex revealed by cryo-EM. (**A**) Representative cryo-EM field. (**B**) Reference-free 2D class averages. (**C**) Views of cryo-EM structure of the complex.

PUBLICATIONS

- Acebrón I, Righetto RD, Schoenherr C, Buhr S, Redondo P, Culley J, Rodríguez CF, Daday C, Biyani N, Llorca O, Byron A, Chami M, Gräter F, Boskovic J, Margaret C Frame, Stahlberg H, Lietha D (2020). Structural basis of Focal Adhesion Kinase activation on lipid membranes. *EMBO J* 39, e104743.
- López-Perrote A, Hug N, González-Corpas A, Rodríguez CF, Serna M, García-Martín C, Boskovic J, Fernandez-Leiro R, Caceres JF, Llorca O. (2020). Regulation of RUVBL1-RUVBL2 AAA-ATPases by the nonsense-mediated mRNA decay factor DHX34, as evidenced by Cryo-EM. *Elife* 9, e63042.
- González-Martínez J, Cwetsch AW, Martínez-Alonso D, López-Sainz LR, Almagro J, Megías D, Boskovic J, Gilabert-Juan J, Graña-Castro O, Pierani A, Behrens A, Ortega S, Malumbres M (2020). Lack of adaptation to centriolar defects leads to p53-independent microcephaly in the absence of Cep135. *BioRxiv*, doi: https://doi.org/10.1101/2020.05.07.082032.