ELECTRON MICROSCOPY UNIT

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Titulado Superior (Advanced Degree)

OVERVIEW

The Electron Microscopy (EM) Unit is a central core facility as well as a research laboratory. It is available to CNIO researchers and the wider research community, providing investigators with instruments and support for Transmission Electron Microscopy analysis. The Unit offers negative staining and cryo-EM specimen preparation techniques for proteins, protein complexes and vesicles. We also give training to regular users on the use of our equipment and provide further guidance regarding specimen preparation.

"Over the past year, the Electron Microscopy Unit has endeavoured to adapt its facility to better meet the needs of the new members of our Programme, particularly in relation to the cryo-EM technique."

RESEARCH HIGHLIGHTS

Over the last decade, cryo-EM has emerged as a key technique for studying how biomolecules function and interact. Our 120-kV Spirit G12 EM, equipped with the TVIPS CMOS detector, has cryo-capabilities that enable sample screening and low resolution analysis of standard biological specimens. For high resolution cryo-EM data collection, CNIO's Structural Biology Programme has been granted access to high-end cryo-EM microscopes at the Electron Bio-Imaging Centre (eBIC) (Oxford, UK) through peer-reviewed Block Allocation Group (BAG) access.

Throughout 2018, the Unit has performed EM experiments with all the research groups from the Structural Biology Programme, as well as with several groups from other CNIO Programmes and outside our Centre. For example, in collaboration with CNIO's Microenvironment and Metastasis Group, we have contributed to the characterisation of circulating extracellular vesicles from the lymph and plasma of melanoma patients. Our data supported their analysis of lymph-circulating extracellular vesicles for the detection of residual disease and its reappearance in melanoma. The most frequent mutation in amyotrophic lateral sclerosis and frontotemporal dementia patients involves mutation of the C*9ORF72* gene, resulting, to a certain extent, in the expression of toxic di peptide arginine repeats (PR). We have evaluated, by electron microscopy, in collaboration with the CNIO Genomic Instability Group, that the presence of $(PR)_{20}$ did not affect the *in vitro* assembly of purified 40S and 60S subunits into 80S particles in the absence of mRNA. Moreover, in collaboration with Dr Iván Ventoso – from the *Centro de Biología Molecular 'Severo Ochoa'* (*CSIC-UAM*) and the *Departamento de Biología Molecular* of the *Universidad Autónoma de Madrid* (*UAM*) – we used electron microscopy to localise gold-labelled eukaryotic initiation factor-4A (eIF4A) in the ribosomal translation initiation complex 48S. Our results have contributed towards the proposal of a topological model of the scanning ribosomal 43S pre-initiation complex. ■

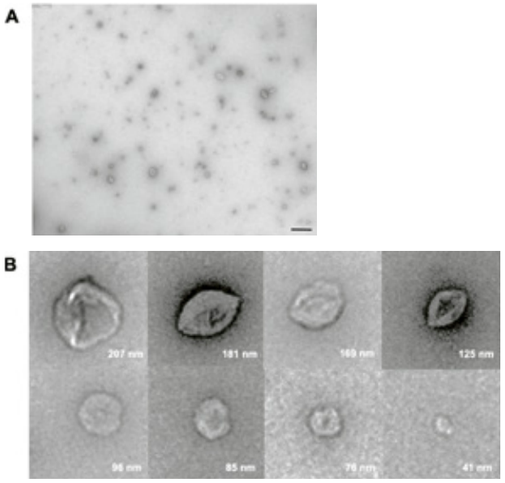

Figure (**A**) Representative electron micrograph of negative stained exosomes derived from the lymph of a melanoma subject, after density gradient centrifugation. Scale bar, 500 nm. (**B**) Analysis of exosome structure and size after density gradient.

PUBLICATIONS

- Toribio R, Díaz-López I, Boskovic J, Ventoso I.(2018).Translation initiation of alphavirus mRNA reveals new insights into the topology of the 48S initiation complex. *Nucleic Acids Res* 46, 4176-4187.
- Lafarga V, Sirozh O, Diaz-Lopez I, Hisaoka M, Zarzuela E, Boskovic J, Jovanovic B, Fernandez-Leiro R, Munoz J, Stoecklin G, Ventoso I, Fernandez-Capetillo O (2018). DNA and RNA binding mediate the toxicity of arginine-rich peptides encoded by C9ORF72 GGGGCC repeats. *BioRxiv*, doi: https://doi.org/10.1101/441808.