Molecular chaperones: an in-depth study of their structures, mechanisms and influence in different cellular processes (CHAPMOL) (PID2022-137175NB-I00)

This proposal intends to shed light in the structure and function of a group of proteins termed molecular chaperones, which are involved in protein homeostasis (proteostasis), as they perform a critical role in protein folding, assembly, disaggregation and degradation (Hartl et al., 2011). They include a large number of well-established families (sHsp, Hsp40, Hsp60 or chaperonins, Hsp70, Hsp90, Hsp100, Hsp110) and, almost as important as the chaperones themselves, a large number of cofactors (cochaperones) that direct the complex between the chaperone and the substrate (the word "substrate" has a enzymatic connotation and the trend in the chaperone community is to use "client protein" or "client"; the latter one will be used from now on) to the right cellular place and to the proper fate, be it folding, assembly or degradation … All these functions executed by chaperones and cochaperones are somehow coordinated and function like a factory assembly line in which a certain chaperone exerts a particular function to the client and transfers it to another chaperone for the next step in the folding/disaggregation/degradation process. The transient complexes formed by chaperones, cochaperones and clients during these processes are particularly suited for their structural analysis by different cryoelectron microscopy (cryoEM) techniques, a branch of electron microscopy that is revolutionizing the structural biology and cell biology fields. This proposal will make use of this and other state-of-the-art techniques (Fig. 1) at its disposal in the unique infrastructure (at the Spanish level) set up at the Centro Nacional de Biotecnología (CNB) cryoEM facility.

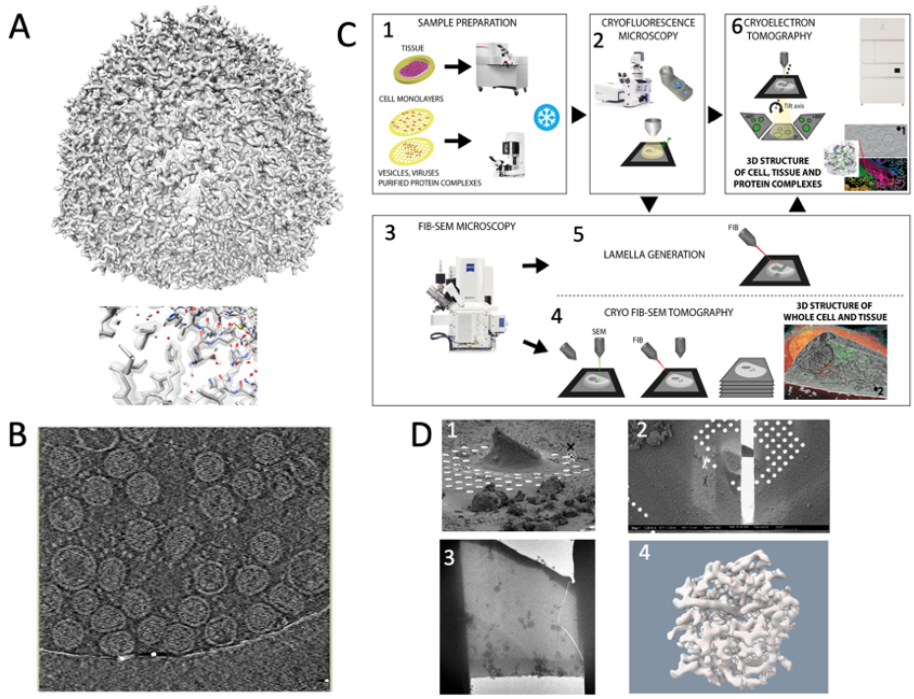

Fig. 1. Examples and brief explanation of some of the techniques described in the proposal. (A) *Top, 3D reconstruction of apoferritin at 1.8 Å resolution carried out at the CNB. Bottom, a detail of the atomic structure of apoferritin into the electron density of the 3D reconstruction.* ***(B)*** *Cryoelectron tomogram presenting a section of TGEV particles.* ***(C)*** *Workflow of cryoCLEM. 1), cell cultures are vitrified using standard pressure or a high-pressure freezing device. 2) the vitrified grids are visualized in a cryofluorescence microscope where the biological events to be characterized (i.e., a particular subcellular region) are tracked. 3) the cryogrids are taken to a cryoFIBSEM. There, either 4) a low-resolution 3D reconstruction can be obtained by iteratively scanning the area using the scanning electron microscopy and thinning it with the Focused Ion Beam (FIB), and merging of the "slices" to obtain a volume, or 5) the cell is thinned to generate lamellas (up to 1000 Å thick) of the region of interest, 6) that can be used to determine the structure of this particular area by cryoET in the 300 kV cryomicroscope.* ***(D)*** *General workflow for microED. 1) The microcrystals of the protein or the macromolecular complex are vitrified in a grid. In many occasions the crystal is still too large for electron diffraction, 2 and 3) and they are thinned to generate lamellas (up to 2000 Å), as described in (C, 3) using the cryoFIBSEM device. 4) Data from the diffraction of the lamellas are used to determine the structure of the protein, in this case a preliminary structural determination of proteinase K (2.7 Å resolution).*