Cryo-CLEM Imaging at CNB-CSIC, Madrid

Jonathan Gabriel Piccirillo[1], Jose Javier Conesa[1], Francisco Javier Chichón[1], Rocio Arranz[1], Maria Teresa Bueno[1], Noelia Zamarreño[1], Jose Maria Valpuesta[1]

[1] Centro Nacional de Biotecnologias—CSIC, Calle Darwin, 3, Madrid, Spain

Introduction: Cryo CLEM the Coolest Kid in Town

Nowadays, there is a growing interest in structural techniques that take advantage of the vitrification of water in biological samples to preserve native conditions. Most of these techniques involve high-resolution methods that aim to solve the structure of purified biological complexes. Nevertheless, these approaches are hindered by analysing the structure without the cellular context. For this reason, the scientific community made a gargantuan effort for the integration of light microscopy techniques in the workflow of the cryo-techniques, allowing them to solve the structure of protein complexes in their native environment (Jun et al., 2019).

A versatil Workflow

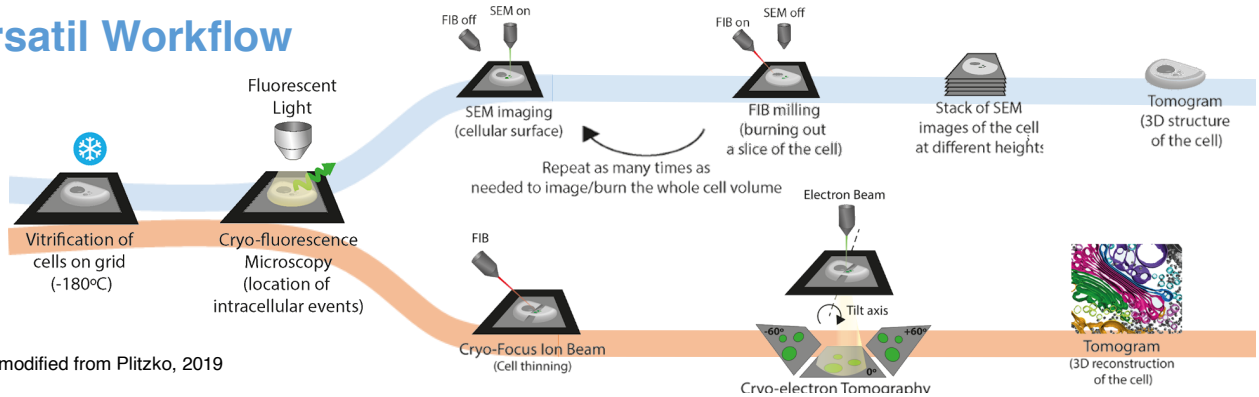
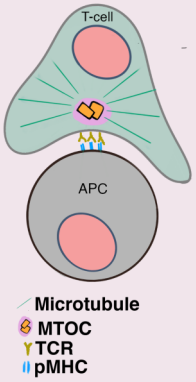


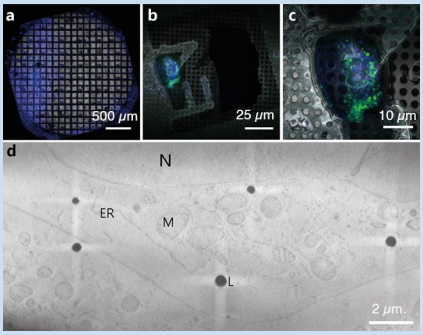
Image modified from Plitzko, 2019

The immune Synapse

By applying cryo-CLEM methodology we aim to study the immune synapse (IS). In the IS dendritic and T-cells stablish cellular communication regions which organization determines the extent of the immune response. We particularly focus on the molecular chaperone CTT that is involved in the regulation of the structure of the immune synapse by the modification of the centrioles and the polarization of tubulin (Martín-Cófreces, 2008)

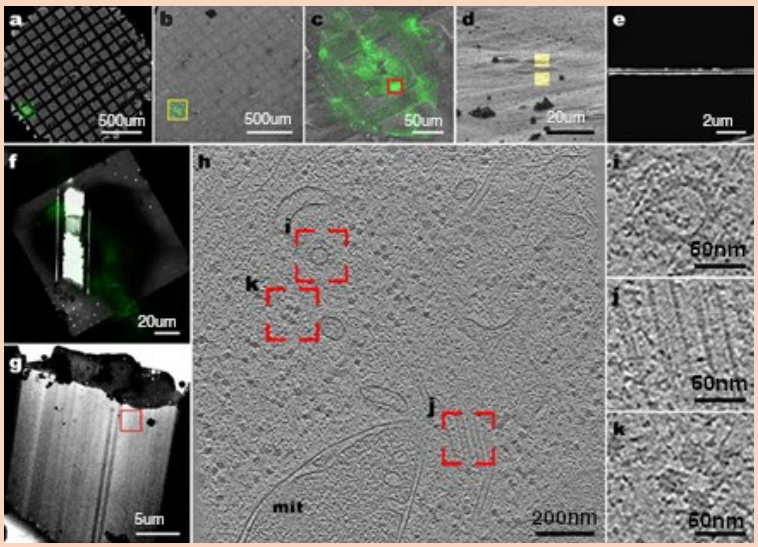


Jurkat—Cryo-CLEM FIB-SEM



Workflow Example of Cryo-Correlative Microscopy with FIB-SEM on activated Jurkat T-cells expressing GFP-EB3. **a.** An overlay of bright field and confocal fluorescence images of a TEM grid. Blue signal, DAPI, Green, GFP-EB3. **b.** magnification of a grid mesh with a Jurkat T-cell. **c.** magnification of the cell present in b. **d.** Tomographic section obtained Cryo-Correlative Microscopy with FIB-SEM on activated Jurkat T-cells expressing GFP-EB3. The resolution of this workflow allows to identify different organules, N, nucleus, ER, Endoplasmatic reticule, M, mitochondria, L, lipid droplets.

Jurkat—Cryo-CLEM with TEM



Workflow Example of Cryo-Correlative Microscopy with TEM on activated Jurkat T-cells expressing GFP-EB3. **a.** An overlay of bright field and confocal fluorescence images of a TEM grid. **b.** overlay of SEM image of the same area presented in a. **c.** magnification of the yellow area in b **d.** SEM image of the area in c, the ablation pattern in yellow and corresponding to the red area in c. **e.** Tateral view of the lamella prepared following the ablation pattern in d. **f.** TEM image with a confocal plane overlay of the corresponding area. **g.** TEM image of the region in f at higher magnification. **h.** virtual section of a 3D volume obtained by cryo-electron tomography of the red area in g, the acquisition area was selected by the obtained information from the confocal microscopy; mit: mitochondria. **i-k.** Magnified view of structures present in h, i, coated vescicles, j, microtubules, k, ribosomes.

REFERENCES

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