Grenoble Alliance for Integrated Structural & Cell Biology

INTERNSHIP PROPOSAL

Institute and Group: IBS group EBEV

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Research project title: Imaging ESCRT-III filaments at HIV-1 budding sites

5 Keywords to describe the project: HIV, ESCRT, Cryo-EM, CLEM, FIB-SEM

Description of the project (aims, experimental techniques, recommended background):

ESCRT-III is an evolutionary conserved protein machinery that mediates membrane remodeling including scission in a very large variety of cellular contexts such as budding of enveloped viruses. A minimal fission machinery composed of CHMP4 (B or A), CHMP3, CHMP2A and the ATPase VPS4 has been proposed based on HIV-1 budding. However, high resolution structure of ESCRT-III at HIV-1 budding sites is still missing because ESCRT-III recruitment is transient and lasts only a few minutes. To overcome these problems for imaging ESCRTs, we combine two approaches. First, we have developed an inducible system that converts CHMP2A into a dominant negative form that inhibits HIV-1 Gag VLP release upon addition of a protease inhibitor. This will be combined with fluorescent detection of the dominant negative form of CHMP2A together HIV-1 Gag budding sites labelled with red fluorescent Gag. Double positive budding sites will be analyzed by Cryo-CLEM (Correlative Light and cryo Electron Microscopy) and/or FIB-SEM (Focused Ion Beam Scanning Electron Microscopy)

enabling precision milling of frozen-hydrated samples, thin lamellae suitable for cryo-electron tomography. The technique will be set up in collaboration with the IBS EM group. The role of the master student will be to develop the CHMP2A inducible dominant negative system with other ESCRT-III proteins such as CHMP3 and CHMP4B. The background of the student should be in molecular cell biology with a strong interest in structural biology.

Justification that the internship's subject fits with the general theme of GRAL:

The project is within the Gral objective of integrated structural biology. Imaging of native ESCRT-III assemblies will provide cellular snapshots of ESCRT-III and will allow their comparison to ESCRT-III assembled i*n vitro* with important implications for the understanding of their function in membrane fission.

Relevant publications of the team:

Ventimiglia, L.N., Cuesta-Geijo, M. A., Martinelli, N., Caballe, A., Macheboeuf, P., Miguet, M., Parnham, I. M., Olmos, Y., Carlton, J. G., Weissenhorn W. and Martin-Serrano, J. (2018) CC2D1B coordinates ESCRT-III activity during the mitotic reformation of the nuclear envelope. *Dev Cell, 47, 547-563.* Effantin, G., A. Dordor, V. Sandrin, N. Martinelli, W.I. Sundquist, G. Schoehn, and W. Weissenhorn. 2013. ESCRT-III CHMP2A and CHMP3 form variable helical polymers in vitro and act synergistically during HIV-1 budding. *Cellular microbiology*. 15:213-226.

Effantin, G., L.F. Estrozi, N. Aschman, P. Renesto, N. Stanke, D. Lindemann, G. Schoehn, and W. Weissenhorn. 2016. Cryo-electron Microscopy Structure of the Native Prototype Foamy Virus Glycoprotein and Virus Architecture. *PLoS pathogens*. 12:e1005721.