UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

**Master's degree in Biology – Chemistry-Biology Department**

# Master 2 internship project
# Year 2019-2020

**Laboratory/Institute:** IBS
**Director:** M. Weissenhorn
**Team:** EBEV
**Head of the team:** M. Weissenhorn

**Name and status of the scientist in charge of the project:**
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**Program of the Master's degree in Biology:**

☐ Immunology, Microbiology, Infectious Diseases ☒ Integrative Structural Biology
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology
☐ Planta International

## Title of the project: Imaging ESCRT protein at Necks

### Objectives (up to 3 lines):

*In vivo*, ESCRT-III-induces membrane constriction and cleavage at membrane sites with a specific morphology: at the neck of buds. The M2 student will use an assay that mimics this membrane shape to reconstitute membrane fission and to address structural mechanisms involved.

### Abstract (up to 10 lines):

The ESCRT complex catalyzes a wide range of membrane remodeling processes at sites with a specific morphology that finish with membrane fission. Based on HIV-1 budding, a minimal ESCRT-III fission machinery has been proposed, composed of CHMP4 (B or A), CHMP3, CHMP2A and the ATPase VPS4 [2]. In vitro reconstitution of ESCRT-III-mediated membrane fission in a physiological-like manner has not been achieved yet. To this aim we successively developped an artificial budding « viruses » that reconstitute membrane with a biomimetic geometry. It is composed of the phage T5 prohead that forms a capsid structure resembling the size of an HIV-1 virion and of Liposomes that behave as plasma membrane. A linker protein that interacted with the T5 capside and the liposome was used to turn T5 into a membrane budding virion. This assay will be used to reconstitute ESCRT-III polymerization at those artificial budding necks and to image membrane constriction and cleavage after addition of Vps4 proteins. It would provide structural and mechanistic data of membrane cleavage, an essential step of HIV infectivity.

### Methods (up to 3 lines):

The student will produce ESCRT-III proteins and add them to artificial budding virus. He will test different concentrations and combinations thereof. The recruitment of ESCRTs will be analyzed by gradient centrifugation of T5-liposome buds followed by SDS-PAGE analysis and imaging by cryo electron microscopy.

### Up to 3 relevant publications of the team:

De Franceschi, N., Alqabandi, M., Miguet, N., Caillat, C., Mangenot, S., Weissenhorn, W., and Bassereau, P. (2019). The ESCRT protein CHMP2B acts as a diffusion barrier on reconstituted membrane necks. J. Cell Sci. *132*, jcs217968.
Maity, S., Caillat, C., Miguet, N., Sulbaran, G., Effantin, G., Schoehn, G., Roos, W.H., and Weissenhorn, W. (2019). VPS4 triggers constriction and cleavage of ESCRT-III helical filaments. Sci. Adv. *5*, eaau7198.
Ventimiglia, L.N., Cuesta-Geijo, M.A., Martinelli, N., Caballe, A., Macheboeuf, P., Miguet, N., Parnham, I.M., Olmos, Y., Carlton, J.G., Weissenhorn, W., et al. (2018). CC2D1B Coordinates ESCRT-III Activity during the Mitotic Reformation of the Nuclear Envelope. Dev. Cell *47*, 547–563.e6.

Requested domains of expertise (up to 5 keywords): Structural biology, recombinant protein production