**Master 2 internship project**

**Year 2024-2025**

**Laboratory/Institute:** IBS **Director:** M. Weissenhorn

**Team:** Entrée et Bourgeonnement des Virus à Enveloppe **Head of the team:** M. Weissenhorn

**Name and status of the scientist in charge of the project:**

Boscheron chercheuse CEA

 **HDR: yes X no ☐**

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**Program of the Master’s degree in Biology:**

**X** Microbiology, Infectious Diseases and Immunology  **X** Structural Biology of Pathogens

**☐** Physiology, Epigenetics, Differentiation, Cancer **☐** Neurosciences and Neurobiology

**Title of the project: High-resolution imaging of ESCRT-III machinery during HIV-1 budding.**

Objectives (up to 3 lines):

Cell line construction for in vivo imaging. Setting up DNA Points Accumulation for Imaging Nanoscale Topography (DNA PAINT) super-resolution microscopy. Imaging of structural organization of ESCRT-III filaments along HIV-1 budding neck.

Abstract (up to 10 lines): HIV-1 requires the cellular ESCRT-III (endosomal sorting complexes required for transport) machinery and the ATPase VPS4 for budding and propagation of infection. The architecture of the native ESCRT-III complex at HIV-1 budding sites is not well understood due to the tiny size of budding necks (~50nm) and the transient nature of ESCRT-III filaments. Here, we propose to leverage DNA PAINT super-resolution microscopy to determine the spatial distribution of ESCRT-III filaments, and the potential transitions between each fiber along the budding neck. We will employ CRISPR-CAS9 genomic editing to tag ESRT-III proteins at their endogenous loci. To establish DNA-PAINT, we will use the catalytically inactive Vps4B E228Q mutant that inhibits HIV-1 budding and strongly enhance ESCRT-III proteins resting time at budding necks. We will then delay HIV-1 budding without fully blocking it using a tool we developed to block ESCRT-III (Wang et al. 2023). Our goal is to deliver in-cell images showing the localization of ESCRT-III within the necks of HIV-1 budding structures, aiming to elucidate their spatial arrangement, structural organization, and interplay during membrane cleavage.

Methods (up to 3 lines):

(i) We will use genomic editing CRISPR-CAS9 techniques to introduce either a FLAG tag or the ALFA-tag, both of which add only a few amino acids to the C-termini of ESCRT-III proteins at their endogenous chromosomal loci. (ii) DNA / Nanobody conjugation with a previously described methods. Iii) DNA-PAINT imaging in VPS4 mutant.

Up to 3 relevant publications of the team:

Azad, K., D. Guilligay, C. Boscheron, S. Maity, N. De Franceschi, G. Sulbaran, G. Effantin, H. Wang, J.P. Kleman, P. Bassereau, G. Schoehn, W.H. Roos, A. Desfosses, and W. Weissenhorn. 2023. Structural basis of CHMP2A-CHMP3 ESCRT-III polymer assembly and membrane cleavage. *Nat Struct Mol Biol*. 30:81-90.

Wang, H.; Gallet, B.; Moriscot, C.; Pezet, M.; Chatellard, C.; Kleman, J.-P.; Göttlinger, H.; Weissenhorn, W.; Boscheron, C., An inducible ESCRT-III inhibition tool to control HIV-1 budding. *Viruses* **2023,** 15, (12), 2289.

Requested domains of expertise (up to 5 keywords):

Biochemistry and microscopy. We warmly welcome serious, curious, and dynamic students !