Grenoble Alliance for Integrated Structural & Cell Biology

GRAL MSc RESEARCH SCHOLARSHIP 2020-2021

RESEARCH INTERNSHIP PROPOSAL

Institute / Group

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Research Project Title

Does banana shaped dimeric ALIX influence Chmp4b polymerization and membrane remodelling?

Description of the project

ESCRT-III is an evolutionary conserved protein machinery that mediates membrane remodelling including scission in a very large variety of cellular contexts such as budding of enveloped HIV-1 viruses. CHMP4b, an ESCRT-III member, is recruited at HIV-1 budding necks through the cellular protein ALIX. It has been proposed that banana shaped dimeric ALIX coordinate CHMP4b polymer assembly and position on membranes. However, experimental system to directly address this hypothesis have been lacking and it is still unknown whether ALIX dimerization entails accurate CHMP4b polymerization. We have recently developped a model membrane system that mimic the shape of a budding viral necks by wrapping reconstituted viral particles with membrane of liposomes. Recombinant ALIX eluted from size exclusion chromatography in dimeric and monomeric forms would be added together with CHMP4b to determine whether CHMP4b assembly and organization at membrane necks discriminate between ALIX status. Further, we will take advantage of an ALIX mutation disabling dimerization. Reads out will be sucrose gradient floatation assay to determine whether monomeric vs dimeric ALIX drive CHMP4b polymerization in model membrane necks and EM (negative staining then cryo) to directly image monomeric vs dimeric ALIX-CHMP4b organization at those sites.

Keywords

HIV-1 budding, ESCRT, ALIX, Cryo-EM

Relevant publications of the team

Maity, S., C. Caillat, N. Miguet, G. Sulbaran, G. Effantin, G. Schoehn, W.H. Roos, and W. Weissenhorn. 2019. VPS4 triggers constriction and cleavage of ESCRT-III helical filaments. Science advances. 5:eaau7198.

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De Franceschi, N., M. Alqabandi, N. Miguet, C. Caillat, S. Mangenot, W. Weissenhorn, and P. Bassereau. 2018. The ESCRT protein CHMP2B acts as a diffusion barrier on reconstituted membrane necks. Journal of cell science. 132.