

INTERNSHIP PROPOSAL

Institute and Group: Institut de Biologie Structurale, Viral Infection and Cancer Group

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Research project title: **Import of HIV-1 Rev into the host cell nucleus**

5 Keywords: HIV-1, nuclear transport, electron microscopy, fluorescence microscopy, FRAP

Description of the project:

Background. The HIV-1 protein Rev is critical for viral replication in the host cell. Rev mediates the export of the replicated viral RNA genome from the nucleus to the cytosol for incorporation into newly formed virions. Rev is imported into the nucleus by the host cell protein Importin β (Imp β), but how Imp β recognizes Rev and delivers it to the nucleus is poorly understood. **Preliminary data.** We have identified point mutations on Rev and Imp β that compromise Imp β /Rev complex formation in vitro. Attempts to crystallize this 110 kDa complex have been unsuccessful; however, preliminary negative-stain and cryo-EM data indicate that the complex is suitable for single particle analysis. **Aims.** This project aims to: (i) determine a low-resolution 3D structure of the Imp β /Rev complex by negative-stain EM and obtain additional image data by cryo-EM (needed to apply for time on the ESRF Krios microscope to collect a high-resolution Volta phase plate dataset); and (ii) investigate the effect of Rev and Imp β point mutations on the nuclear localisation and transport dynamics of Rev in human cells by fluorescence microscopy and FRAP, respectively. **Techniques.** Protein purification, negative-stain and cryo-EM, cell culture, fluorescence microscopy, FRAP. (Research in structural and cell biology will be supervised by C. Petosa and D. Skoufias, respectively, whose teams merged in Sept. 2018.) **Recommended background.** Biochemistry, structural biology and/or cell biology. The project will provide the student with experience in protein biochemistry, single particle EM analysis and cellular imaging.

Justification that the internship's subject fits with the general theme of GRAL (3 lines):

This project aims to integrate knowledge on the structural basis and dynamics of HIV-1 Rev nuclear import in the cellular context. It is thus in line with GRAL's focus on integrated structural and cell biology within the theme of host-pathogen interactions.

Relevant publications of the team (3 max):

We do not yet have publications concerning this HIV-1 Rev project, which derives from early work described in publication 1.

1. Cingolani G, [Petosa C](#), Weis K, Müller CW. (1999) Structure of Importin β bound to the IBB domain of Importin α . *Nature* 399:221-229.

2. Mietton F, Ferri E, Champlébourg M, Zala N, Maubon D, Zhou Y, Harbut M, Spittler D, Garnaud C, Chauvel M, d'Enfert C, Kashemirov BA, Hull M, Cornet M, McKenna CE*, Govin J*, [Petosa C*](#). (2017) Selective BET bromodomain inhibition as an antifungal therapeutic strategy. *Nature Comm.*, 8:15482.

3. Garcia-Saez I, Menoni H, Boopathi R, Shukla MS, Soueidan L, Noirclerc-Savoye M, Le Roy A, Skoufias DA, Bednar J*, Hamiche A*, Angelov D*, [Petosa C*](#), Dimitrov S*. (2018) Structure of an H1-bound 6-nucleosome array reveals an untwisted two-start chromatin fiber conformation. *Mol Cell*. 72:902-915.