Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

Supervisor(s):
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Host laboratory:
IBS https://www.ibs.fr/

Host group/team:
Epigen: Epigenetics & molecular pathways

Title of the M2 research internship:

Nuclear import of HIV-1 Rev : a comprehensive analysis of molecular interactions with host cell proteins

Project summary:

Background: The HIV-1 regulatory protein Rev is a nucleocytoplasmic shuttling protein essential for viral replication. Rev mediates the specific nuclear export of viral RNA transcripts needed to synthesize new viral particles. Rev is synthesized in the early phase of infection and is imported into the nucleus by direct interaction with host cell proteins Importin β (Impβ) and Nucleophosmin (NPM1) (1-3). The interaction between Rev and Impβ is essential for Rev nuclear import (4), while the Impβ-dependent interaction between Rev and NPM1 enhances the nuclear import of Rev (5). However, despite the crucial role of Impβ and NPM1 in the viral infection cycle (4,6), the molecular basis of these interactions remains poorly understood.
Preliminary results: We recently characterised the interaction between Impβ and Rev through extensive biochemical, biophysical and computational analyses, allowing us to report the first 3D structural model of the Impβ/Rev complex (7). Preliminary data by electron microscopy (negative stain and cryo-EM) indicate that this complex is suitable for single particle analysis. Additionally, we identified NPM1 domains that interact directly with Rev as well as with Impβ, suggesting the existence of a ternary complex that may play a regulatory role during the viral infection cycle.
Aims: The overall project aims to (i) determine the EM structures of Rev in complex with Impβ and NPM1, (ii) characterise the Rev/NPM1 interaction by a set of biochemical and biophysical techniques, (iii) study the dynamics of Impβ/Rev/NPM1 complex formation by cross-linking/mass spectrometry analyses and (iv) investigate the effect of Rev, Impβ and NPM1 point mutations on Rev nuclear import in human cells by fluorescence microscopy.
According to his/her interest and background, the student will participate in one or more of these objectives.
Techniques: Protein purification, fluorescence polarization, ITC, SEC-MALLS, mass photometry, crosslinking, native mass spectrometry, cell culture, fluorescence microscopy, negative-stain, cryo-EM, NMR.

Keywords:
HIV-1, protein complex, structural biology

Relevant publications of the team:

Spittler D, Indorato RL, Boeri Erba E, Delaforge E, Signor L, Harris SJ, Garcia-Saez I, Palencia A, Gabel F, Blackledge M, Noirclerc-Savoye M, Petosa C. 2022. Binding stoichiometry and structural model of the HIV-1 Rev/importin β complex. Life Sci Alliance 5. https://doi.org/10.26508/lsa.202201431

Ben Fadhel N, Signor L, Petosa C, Noirclerc-Savoye M. 2019. Phosphomimetic mutations modulate the ability of HIV-1 Rev to bind human Importin β in vitro. Matters (ISSN: 2297-8240). https://hal.univ-grenoble-alpes.fr/view/index/docid/3605154

Coscia F, Estrozi LF, Hans F, Malet H, Noirclerc-Savoye M, Schoehn G, Petosa C. 2016. Fusion to a homo-oligomeric scaffold allows cryo-EM analysis of a small protein. Sci Rep 6:30909. https://doi.org/10.1038/srep30909

Cingolani G, Petosa C, Weis K, Muller CW. 1999. Structure of importin-beta bound to the IBB domain of importin-alpha. Nature 399:221-9. https://doi.org/10.1038/20367

References

1. Fankhauser et al. 1991 https://doi.org:10.1128/mcb.11.5.2567-2575.1991
2. Li et al. 2005 https://doi.org:10.1038/sj.cr.7290370
3. Nouri et al. 2015 https://doi.org:10.1371/journal.pone.0143634
4. Truant & Cullen 1999 https://doi.org:10.1128/MCB.19.2.1210
5. Szebeni et al. 1997 https://doi.org:10.1021/bi9627931
6. Passos-Castilho et al. 2018 https://doi.org:10.1016/j.virol.2017.12.021
7. Spittler et al. 2022 https://doi.org:10.26508/lsa.202201431