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

Supervisor(s):
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Host laboratory:
Lab : IBS

Host group/team:
I2SR/Timmins(GenOM)

Title of the M2 research internship:

Elucidating the role of NPM1 in HIV-1 replication: a comprehensive analysis of molecular interactions with the HIV-1 Rev protein using integrative structural biology approaches

Project summary:

Background: Nucleophosmin (NPM1) is a multifunctional human protein involved in various cellular processes, such as ribosome biogenesis, cell cycle regulation, DNA repair and apoptosis, as well as in the replication of certain viruses, including HIV-1. The HIV-1 regulatory protein Rev is a nucleocytoplasmic shuttling protein essential for viral replication. Rev is synthesized during the early phase of infection and must be imported into the nucleus to mediate the specific nuclear export of viral RNA transcripts required for the synthesis of new viral particles. NPM1 enhances the solubility and mobility of Rev, accelerating its nuclear import in a pathway-dependent manner involving the human importin protein Impβ, which is essential for the nuclear import of Rev. Given NPM1's crucial role in the nucleolar localisation of Rev and HIV-1 replication, it represents an attractive target for inhibiting viral replication. However, despite the importance of NPM1, the molecular basis and dynamics of the NPM1/Rev interaction across different cellular compartments, as well as its link with Impβ, remain poorly understood and have yet to be fully characterised. Our goal is thus to elucidate the molecular and structural determinants that enable the formation and function of protein complexes involving NPM1, Rev and ImpB using an integrative approach, combining biophysical interaction assays, high-resolution structural techniques and advanced cellular imaging methodologies.

Preliminary results: We have begun to characterize the interaction between NPM1 domains and Rev through biochemical and biophysical analyses. Preliminary data obtained by cryo-electron microscopy (cryo-EM) indicate that NPM1/Rev complex is suitable for single-particle analysis, while initial interaction experiments performed by NMR have identified protein domains involved in the interaction. Furthermore, we observed changes in protein interactions when NPM1, Rev, and Impβ are co-incubated *in vitro*, suggesting the existence of multiple protein complexes that may play a regulatory role during the viral replication cycle.

Aims: The aim of this internship is to identify and characterise the interactions between NPM1, Rev and ImpB using biochemical, biophysical and structural approaches. The intern will produce and purify these three proteins, enabling the reconstitution of protein complexes for structural studies, and their characterisation using biochemical and biophysical methods.

Techniques: Gene mutagenesis, protein purification, biophysical tests for protein-protein interactions (size-exclusion chromatography (SEC), isothermal titration calorimetry (ITC), mass photometry, crosslinking,…), cryo-EM, NMR.

Keywords:
NPM1, Rev, 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