Funded PhD project in Integrated Structural & Cell Biology in Grenoble, France

Title of the PhD project:
Flu-nucleocapsid: Dynamic of influenza virus nucleocapsid assembly

PhD supervisors:
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Host laboratory:
Institut de Biologie Structurale, VRM group

Project summary:
Influenza virus is a negative-sense single-stranded segmented RNA virus. The eight RNA segments (vRNA) of its genome are individually encapsidated into ribonucleoprotein (RNP) complexes that are central to the viral life cycle and for adaptation to new host species. Each RNP is constituted by a single RNA polymerase bound to the complementary vRNA termini and multiple copies of the viral nucleoprotein (NP). In the recent years, important progresses have been made on the influenza polymerase structural analysis. On the opposite, the nucleocapsid (the complex between NP and the vRNA) is almost unknown, limited to X-Ray structures of RNA-free NPs and low resolution cryo-EM reconstructions. By deciphering NPs, T. CREPIN and G. SCHOEHN have shown that nucleocapsid-like particles can be reconstituted *in vitro* by mixing recombinant NP with small RNA probes in precise salt conditions (1). These particles, similar to RNPs extracted from the virus, constitute promising tools to understand the encapsidation mechanism of influenza genome. By combining biochemical, biophysical and electron microscopy technics, the present project aims to optimize the conditions for controlling the assembly of these nucleocapsid-like particles and to obtain the first high-resolution cryo-EM structure for the complex between influenza NP and the RNA. The structural analysis will be transposed at the cellular/viral level for validation through external collaborations. These data are crucial to understand (i) the encapsidation mechanism of influenza genome, (ii) the RNA-dependent assembly of the eight RNPs during the budding process for reconstituting the fully functional progeny virions. They represent also new future direction to design potential new antivirals.

Required skills:
The PhD candidate should have a strong technical background in biochemistry with good knowledge in biophysics and structural biology technics (*i.e.* X-ray crystallography and EM). RNA manipulation would be an additional criterion.

Student role:
The PhD candidate will be in charge of the operational aspect of the project. Using molecular biology approaches, she/he will conceive the necessary tools. The proteins will be expressed, purified and characterized by several biophysics methods. Once done, the optimization of the conditions to obtain homogeneous influenza nucleocapsid-like particles suitable for cryo-EM will be carried out, combining biochemical, biophysical and EM technics. This stage will be the pivotal point to shift to the structural analysis by cryo-EM, conducted (under supervision) by the PhD candidate. She/he will be involved in the structure deciphering for cellular/viral validation as well as the writing of the publication(s) and oral presentation during meetings/congresses.

Keywords:
influenza virus, dynamic of protein-RNA complexes, assembly, nucleocapsid, electron microscopy

Relevant publications of the team:

- Labaronne A, Swale C, Monod A, Schoehn G, Crépin T and Ruigrok RW (2016) Binding of RNA by the nucleoproteins of influenza viruses A and B. *Viruses*, 8, 247; doi:10.3390/v8090247.
- Donchet A, Vassal-Stermann E, Gérard FCA, Ruigrok RWH and Crépin T (2020) Differential behaviours and preferential bindings of influenza nucleoproteins on importins-α. *Viruses*, 12, 834. doi:10.3390/v12080834.
- Arragain B, Reguera J, Desfosses A, Gutsche I, Schoehn G, Malet H (2019) High resolution cryo-EM structure of the helical RNA-bound Hantaan virus nucleocapsid reveals its assembly mechanisms. *Elife*, 8:e43075. doi: 10.7554/eLife.43075.
- Donchet A, Oliva J, Labaronne A, Tengo L, Miloudi M, Gérard FCA, Mas C, Schoehn G, Ruigrok RW, Ducatez M and Crépin T (2019) The structure of the nucleoprotein of Influenza D shows that all *Orthomyxoviridae* nucleoproteins have a similar NP_{CORE}, with or without a NP_{TAIL} for nuclear transport. *Sci Rep*, 9:600. doi: 10.1038/s41598-018-37306-y.
- Gutsche I, Desfosses A, Effantin G, Ling WL, Haupt M, Ruigrok RW, Sachse C, Schoehn G (2015) Structural virology. Near-atomic cryo-EM structure of the helical measles virus nucleocapsid. *Science*, 348(6235):704-7. doi: 10.1126/science.aaa5137.