

INTERNSHIP PROPOSAL

Institute and Group: Institut de Biologie Structurale, Electron Microscopy and Method group directed by Dr. Guy Schoehn

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Research project title: Structural characterization of Hantaan virus replication complexes by single particle cryo-electron microscopy and tomography

5 Keywords to describe the project: electron microscopy, negative strand RNA virus, nucleocapsid, RNA polymerase, replication.

Description of the project (aims, experimental techniques, recommended background):

Hantaviruses are negative stranded RNA viruses belonging to the *Bunyavirales* order. The *Hantaviridae* family encompasses several life-threatening viruses such as Hantaan virus that is causing haemorrhagic fever with renal syndrome. Neither vaccine or antivirals are currently available to counteract it. In this context, we focus our research on two critical steps of Hantaan virus cycle: replication and transcription. These reactions are carried out by the multifunctional viral polymerase which uses nucleocapsids as templates. Viral nucleocapsids are formed by viral RNA segments enwrapped with multiple viral nucleoproteins. We have significant preliminary results on the project, including high quality cryo-EM data of Hantaan virus nucleocapsid and a collaborator is able to express and purify Hantaan virus polymerase. We have also access to Tula virus sample (Tula virus belongs to the *Hantaviridae* family but is non-pathogenic to humans).

The internship will consist in expression, purification and biochemical characterization of either the polymerase or the nucleocapsid/polymerase complex, followed by 3D structural determination by single-particle electron microscopy (negative stain and cryo-EM). Electron tomography will also be used for characterization of replication complexes in fixed Tula hantavirus particles.

The intern will benefit from the EM expertise in the group and will have access and training to the IBS state-of-the-art EM facility, notably a Thermofisher Glacios microscope equipped with a Falcon II direct electron detector and soon with Gatan K2 direct electron detector. Students from either ISB, IMID and MCMV Masters have the requested background and are encouraged to apply if they are interested.

Justification that the internship's subject fits with the general theme of GRAL:

This project perfectly fits GRAL scheme as it corresponds to an integrated structural analysis of essential components of a pathogen, ranging from high resolution analysis of individual proteins to structural analysis in the viral context. It will use top-end electron microscopes available and maintained in the Electron Microscopy and Method group of the Structural Biology Institute (IBS).

Relevant publications of the team:

1. Arragain B, Reguera J, Desfosses A, Gutsche I, **Schoehn G, Malet H**. *eLife*. 2019 Jan 14;8. doi: 10.7554/eLife.43075.
2. Gerlach P¹, **Malet H**¹, Cusack S, Reguera J. Divergent segmented negative strand viral polymerases have the same architecture and mode of regulation by vRNA. 2015. *Cell*. Jun 4;161:1267-79 ¹co-first author
3. Gutsche I, Desfosses A, Effantin G, Ling WL, Haupt M, Ruigrok RW, Sachse C, **Schoehn G**. Structural virology. Near-atomic cryo-EM structure of the helical measles virus nucleocapsid. *Science*. 2015 May 8;348(6235):704-7.