

**Supervisor(s):**

Name : H       MALET

E-Mail Address : helene.malet@ibs.fr

**Host laboratory:**

Lab : Structural Biology Institute/Institut de Biologie Structurale

**Host group/team:**

Electron Microscopy and Method Group/Groupe Microscopie   lectronique et M  thodes

**Title of the M2 research internship:**

Characterization of Rice Hoja Blanca virus polymerase using cryo-electron microscopy and functional assays

**Project summary:**

The *Bunyaviricetes* class of segmented negative-strand RNA viruses (sNSVs) includes over 500 species across fifteen families. Spread globally, bunyaviruses pose a significant threat to human health and cause harm to livestock and crops. For this internship, we will focus on a plant-infecting virus, *Rice hoja blanca virus* (RHBV), from the *Phenuiviridae* family. Rice is the primary staple food for more than half of the world's population, and rice infections caused by phenuiviruses lead to major yield losses and significant economic impacts. The aim of this project is to characterize the structure and activities of RHBV polymerase, which performs essential functions during the viral cycle: (i) transcription by *cap-snatching*, which generates viral mRNA, (ii) *de novo* replication, which duplicates the viral genome, (iii) innate immune response evasion, which counteracts the plant's defense mechanisms against viral infection. The M2 intern will be involved in the expression of RHBV polymerase (330 kDa) in insect cells and its purification using protocols already optimized in the lab. He/She will learn how to use electron microscopes (EM) maintained by our group to collect negative-stain and cryo-EM data. Additionally, the intern will perform cryo-EM image analysis of RHBV polymerase, aiming to determine its 3D structure at high resolution. Depending on the progress of other parts of the project, he/she may also characterize the binding of viral RNA and capped mRNA primers to RHBV polymerase, which are prerequisites for future replication and transcription assays.

**Keywords:**

Bunyavirus, Plant virus, Polymerase, Replication, Transcription, Innate immune response evasion.

**Relevant publications of the team:**

1. Arragain, B., Effantin G, Gerlach P, Reguera J, Schoehn G, Cusack S, Malet H. Pre-initiation and elongation structures of full-length La Crosse virus polymerase reveal functionally important conformational changes. *Nat Commun* **11**, 3590 (2020).
2. Arragain, B., Durieux Trouilleteon Q, Baudin F, Provaznik J, Azevedo N, Cusack S, Schoehn G, Malet H Structural snapshots of La Crosse virus polymerase reveal the mechanisms underlying Peribunyaviridae replication and transcription. *Nat Commun* **13**, 902 (2022).
3. Durieux Trouilleteon, Q., Barata-Garc    , S., Arragain, B., Reguera, J. & Malet, H. Structures of active Hantaan virus polymerase uncover the mechanisms of Hantaviridae genome replication. *Nat Commun* **14**, 2954 (2023).
4. Durieux Trouilleteon, Q., Housset, D., Tarillon, P., Arragain, B. & Malet, H. Structural characterization of the oligomerization of full-length Hantaan virus polymerase into symmetric dimers and hexamers. *Nat Commun* **15**, 2256 (2024).
5. Malet, H., Williams, H. M., Cusack, S. & Rosenthal, M. The mechanism of genome replication and transcription in bunyaviruses. *PLoS Pathog* **19**, e1011060 (2023).