**Master 2 internship project**

**Year 2024-2025**

**Laboratory/Institute:** Institut de Biologie Structurale **Director:** Winfried WEISSENHORN

**Team:** Viral Replication Machines **Head of the team:** Marc JAMIN

**Name and status of the scientist in charge of the project:** Wim BURMEISTER, Professor **HDR: yes 🗹 no ☐**

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**Program of the Master’s degree in Biology:**

**☐** Microbiology, Infectious Diseases and Immunology **🗹** Structural Biology of Pathogens

**☐** Physiology, Epigenetics, Differentiation, Cancer **☐** Neurosciences and Neurobiology

**Title of the project: Structural and functional study of the telomere-binding proteins of vaccinia virus**

Objectives (up to 3 lines):

The mechanism of poxvirus DNA replication, our research subject, is not understood. We suppose that the telomere structure at the circularized extremities of the linear dsDNA genome is important in the initiation of viral DNA replication and we want to work on its structure and the structure of the associated proteins.

Abstract (up to 10 lines):

With the 2022 epidemic outbreak of mpox, poxviruses got into the headlines. A safe model system is vaccinia virus, which is 98 % identical to mpox at the amino acid level regarding the proteins involved in DNA replication. The dsDNA genome of poxviruses is circularized at the extremities with loops surrounded by a ≈35 bp telomere region with imperfect base-pairing. Three proteins present also in viral particles are associated with the telomeres and may also be involved in genome packaging. It is likely that the viral genome replication starts at the telomere as there is no proven origin of replication. In our team, we produced the telomere-binding proteins in bacteria and in the baculovirus-insect cell system and obtained oligomeric assemblies suitable for a structure determination by cryo-electron microscopy, possibly also in complex with telomere DNA. We expect that this project will give new insights in the interplay between DNA replication, genome packaging and uncoating of the poxvirus genome in the infected cell.

Methods (up to 3 lines):

Protein production in the baculovirus-insect cell system or in *E. coli*. Affinity chromatography. Charac-terization of interactions with DNA by gel shift (EMSA) or biolayer interferometry (BLI). Sample preparation for negative stain-EM and cryo-EM. Cryo-EM data processing and reconstruction of the structure.

Up to 3 relevant publications of the team:

Burmeister, W. P., Boutin, L., Balestra, A. C., Gröger, H., Ballandras-Colas, A., Hutin, S., Kraft, C., Grimm, C., Böttcher, B., Fischer, U., Tarbouriech, N. & Iseni, F. (2024) Structure and flexibility of the DNA polymerase holoenzyme of vaccinia virus. Plos Path. <https://doi.org/10.1371/journal.ppat.1011652>.

Hutin, SL, Ling, W.L., Tarbouriech, N., Schoehn, G., Grimm, C., Fischer, U. & Burmeister, W.P. (2022) The Vaccinia Virus DNA Helicase Structure from Combined Single-Particle Cryo-Electron Microscopy and AlphaFold2 Prediction. Viruses 14 (10). [doi/10.3390/v14102206](https://doi.org/10.3390/v14102206).

Tarbouriech, N., Burmeister, W.P., Bersch, B. & Iseni, F. Le complexe de réplication des poxvirus : cible potentielle de molécules antivirales. (2024) Virologie 28 (1): 23‑35. <https://doi.org/10.1684/vir.2024.1033>.

Requested domains of expertise (up to 5 keywords): structural biology, protein production, protein purification, biophysical techniques, basic computing skills.