**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: Analysis of the processivity of the poxvirus DNA polymerase**

Objectives (up to 3 lines):

Recently, structures of the poxvirus DNA polymerase with its processivity factor in complex with different DNA templates, primers and nucleotides have been published by several groups. Still, the real contribution of the processivity factor to the processive DNA synthesis on long templates needs to be established.

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. Although the structure of the polymerase holoenzyme composed in vaccinia virus of the polymerase E9 and the processivity factor A20-D4 is now quite well understood, enzymatic activities have only been studied on very short templates reflecting poorly the context of processive DNA synthesis. We can produce the different proteins involved in DNA replication and developed a system, which allows to study the activity on templates measuring several hundreds of bases in order to address a number of open questions about the role and the functioning of the processivity factor and other proteins such as a DNA-binding protein H5 and the single-stranded DNA binding protein I3.

Methods (up to 3 lines):

Protein production in the baculovirus-insect cell system or in *E. coli*. Protein purification by Ni-NTA or size exclusion chromatography. Generation of long single-stranded DNA templates by asymmetric PCR, Polymerase activity assays based of fluorescently labelled primers. Analysis by polyacrylamide gel electrophoresis.

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): protein production, protein purification, molecular biology, basic computing skills.