UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

**Master's degree in Biology – Chemistry-Biology Department**

# Internship project Master 2
# Year 2018-2019

**Laboratory/Institute:** IBS
**Group:** VRM
**Director:** Winfried WEISSENHORN
**Head of the group:** Marc JAMIN

**Name and status of the scientist in charge of the project:** BURMEISTER Wim, Professor
**Address:** IBS – CIBB, 71 avenue des Martyrs, CS 20192, 38044 Grenoble Cedex 9
**Phone:** +33 (0) 457 42 87 41 **e-mail:** wim.burmeister@ibs.fr **HDR: yes** ☑ **no** ☐

**Program the Master's degree in Biology:**

☐ Neurosciences and Neurobiology
☐ Immunology, Microbiology, Infectious Diseases
☒ Integrative Structural Biology
☐ Physiology, Epigenetics, Development, Differentiation

**Title of the project:**

**Structure determination of the DNA polymerase complex of vaccinia virus**

Objectives (up to 3 lines):

The aim is to determine the structure of the vaccinia virus polymerase complex composed of the catalytic subunit E9, the processivity factor A20 and the uracil-DNA glycosylase D4. The complex is produced using the baculovirus system and will be studied cryo- electron microscopy and x-ray crystallography.

Abstract (up to 10 lines):

Before its eradication, smallpox has been the most devastating disease of humanity. Nowadays, there is a risk of an introduction of an orthopoxvirus from an animal reservoir into the human population as poxvirus circulate widely at the level of farm animals and wild rodents. The viral DNA polymerase is one of the key proteins for viral replication and is also the target of several antivirals. We determined the structure of the poxvirus DNA polymerase E9 by x-ray crystallography in the DNA-free form. In order to be processive, E9 has to be incorporated into the E9-A20-D4 holoenzyme complex whose high-resolution structure is still largely unknown, in particular the one of A20. We want to determine the high-resolution structure of the complex by single-particle cryo-electron microscopy or by x-ray crystallography. The structural information on the complex is used in our group in order to design optimized peptides which will interfere with the assembly of the subunits.

Methods (up to 3 lines):

E9-A20-D4 is produced in the baculovirus - insect cell system and purified by affinity and size exclusion chromatography. The structure of the complex will be determined by single-particle cryo-electron microscopy or protein crystallography. Components and fragments of the complex can be expressed in *E. coli.*

Up to 3 relevant publications of the team:

1. Tarbouriech, N., Ducournau, C., Hutin, S., Mas, P. J., Man, P., Forest, E., Hart, D.J., Peyrefitte, C. N., Burmeister, W. P., & Iseni, F. The vaccinia virus DNA polymerase structure provides insights into the mode of processivity factor binding. Nat. Comm. Doi: 10.1038/s41467-017-01542-z (2017).
2. Burmeister, W.P., Tarbouriech, N., Fender, P., Contesto-Richefeu, C., Peyrefitte, C.N. & Iseni, F. Crystal structure of the vaccinia virus uracil DNA-glycosylase in complex with DNA. J Biol. Chem. 290, 17923-17934 (2015).
3. Hutin, S., Ling, W. L., Round, A., Effantin, G., Reich, S., Iseni, F., Tarbouriech, N., Schoehn, G. & Burmeister, W. P. Domain organization of vaccinia virus helicase-primase D5.J. Virol. 90, 4604-4613 (2016).

Requested domains of expertise (up to 5 keywords):

Protein expression, protein purification, structural biology.