UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Internship project Master 2
Year 2017-2018

Laboratory/Institute: IBS
Team: VRM – poxvirus team
Director: Winfried WEISSENHORN
Head of the team: Wim BURMEISTER

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@cegetel.net

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:

Analysis of different structural states of the DNA polymerase E9 of vaccinia virus

Objectives (up to 3 lines):

Understand the different functional states of vaccinia virus E9 DNA polymerase and the action of resistance mutations directed against different polymerase inhibitors using biophysical techniques and macromolecular crystallography.

Abstract (up to 10 lines):

Before its eradication, smallpox has been the most devastating disease of humanity. With an almost non-existing vaccination coverage of the human population nowadays, there is a risk of an introduction of an orthopoxvirus into the human population from an animal reservoir as poxvirus circulate widely at the level of farm animals and wild rodents. In this context, we determined the structure of the poxvirus DNA polymerase E9 by X-ray crystallography in the DNA-free apo form. But there are, as for other B family polymerases, 3 different DNA-bound conformations:
- the complex with a DNA template and a complementary strand in elongation mode
- the complex in elongation mode with a bound nucleotide ready to be incorporated
- the complex with a template DNA and a complementary strand in edition mode
which we would like to study as they are relevant for drug design and antiviral resistance.

Methods (up to 3 lines):

E9 is produced in the baculovirus - insect cell system and purified by affinity and size exclusion chromatography. DNA-protein complexes are crystallized for crystallographic analysis. E9-DNA interactions are analyzed by SPR, fluorescence anisotropy and SAXS. Mutants are generated in *E. coli*.

Up to 3 relevant publications of the team:

1. Tarbouriech, N., Ducournau, C., Hutin, S., Mas, P.J. Man, P., Forest, P., Hart, D.J., Peyrefitte, C. N., Burmeister, W.P. & Iseni, F. High-resolution structure of the vaccinia virus E9 protein: Insight into the structural organization of the DNA polymerase holoenzyme. Accepted with minor modification.
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):

Molecular biology, protein expression, protein purification, biophysical techniques, protein crystallography