

## INTERNSHIP PROPOSAL

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**Research project title:** Analysis of the different structural states of the DNA polymerase E9 of vaccinia virus

### **5 Keywords to describe the project:**

DNA polymerase, X-ray crystallography, protein-DNA complex, poxvirus, large DNA virus

### **Description of the project (aims, experimental techniques, recommended background):**

Before its eradication, smallpox has been the most infectious 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 poxviruses 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 which is the base of the development of new antivirals and the understanding of resistance mutations. E9 will show, as other B family polymerases do, 4 different 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
- the apo form of the polymerase without the presence of an oligonucleotide.

So far, we have solved only the structure of the apo form, but we want to characterize and crystallize several E9-DNA complexes. The production of E9 in the baculovirus - insect cell system, its purification, biophysical characterization of E9 – DNA complexes and their crystallization will be the object of the proposed project.

### **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. In preparation, will be published prior to the start of the fellowship.
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. *Virology* **90**, 4604-4613 (2016).