Grenoble Alliance for Integrated Structural & Cell Biology

INTERNSHIP PROPOSAL

Institute and Group:

Institut de Biologie Structurale, VRM group

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Research project title: Activity of the vaccinia virus DNA polymerase holoenzyme

5 Keywords to describe the project: DNA polymerase, poxvirus, fluorescent labels, enzyme assay, baculovirus expression

Description of the project (aims, experimental techniques, recommended background):
Vaccinia virus replication takes places in the cytosol independently of host proteins. Together with its processivity factor, composed of the uracil-DNA glycosylase (UNG) D4 and a structural protein A20, E9 forms the 190 kDa polymerase holoenzyme E9-A20-D4. The high-resolution structure of the E9 polymerase subunit in its apo-form has been determined recently by us and showed the close relation to other family B polymerases, whereas the processivity factor is unique.
A biochemical study of the activity of the holoenzyme will give important insights into the organization of the replication fork of poxviruses and may also explain the unique activity of E9 in recombination. So far, we studied only the activity of the isolated catalytic E9 subunit.
The holenzyme will be produced in the baculovirus - insect cell system using an established protocol. Using mainly gel electrophoresis- and fluorescence-based techniques, the DNA polymerase activity on large DNA substrates will be studied, furthermore questions of the binding to DNA substrates of different length may be addressed using surface plasmon resonance. Continuous assays of polymerase activity in 96-well format may also be developed. Long-term aims are the lay-out of the foundations for the structural characterization of the holoenzyme-DNA interaction by cryo-electron microscopy.

Justification that the internship's subject fits with the general theme of GRAL:

This Master2 project is a brick in the larger project on the structure and dynamics of the vaccinia virus polymerase holoenzyme by cryo-electron microscopy which will also provide more structural information for the design of inhibitors directed against its protein-interfaces.

Relevant publications of the team:

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*. **8**, 1455 - 1466 : Doi: 10.1038/s41467-017-01542-z (2017).

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).

Sèle, C., Gabel, F., Gutsche, I., Ivanov, I., Burmeister, W.P., Iseni, F. & Tarbouriech, N. Low-Resolution Structure of the Vaccinia Virus DNA Replication machinery. *J. Virol.* **87**, 1679-1689 (2012).