UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Internship project Master 2
Year 2020-2021

Laboratory/Institute: IBS
Group: VRM
Director: Winfried WEISSENHORN
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Program the Master's degree in Biology:

☐ Neurosciences and Neurobiology
☒ Integrative Structural Biology
☐ Planta International
☐ Immunology, Microbiology, Infectious Diseases
☐ 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 will be produced using the baculovirus system in order to get a preparation for first pictures by cryo-electron microscopy.

Abstract (up to 10 lines):

Before its eradication, smallpox has been the most devastating disease of humanity. Nowadays, orthopoxviruses are a class of viruses widespread in animal reservoirs with the potential of a pandemic upon an introduction into the human population as occurred recently with the SRAS-2 coronavirus. The viral DNA polymerase is one of the key proteins for viral replication and also target of several antivirals. We solved 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 with still unknown high-resolution structure. We want to determine this structure by single-particle cryo-electron microscopy (cryo-EM). The complex will be produced by an existing baculovirus expressing the 3 partner proteins, whereas the protocol of the sample preparation for cryo-EM still has to be established.

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 conditions for making grids for cryo-EM have to be established in order to be able to determine the structure by single-particle cryo-EM.

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, computer literacy, structural biology, cryo-electron microscopy.