UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2022-2023

Laboratory/Institute: Institut de Biologie Structurale **Director:** Winfried WEISSENHORN
Team: Viral Replication Machines **Head of the team:** Marc JAMIN

Name and status of the scientist in charge of the project: Wim BURMEISTER, Professor **HDR: yes** ☑ **no** ☐
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Program of the Master's degree in Biology:

☐ Microbiology, Infectious Diseases and Immunology ☑ Structural Biology of Pathogens
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology

Title of the project: Characterization of interactions of poxvirus replication complexes with DNA and nucleotide substrates

Objectives (up to 3 lines):

The aim is the characterization of the interactions of DNA substrates with complexes of the poxvirus DNA replication machinery in view of the determination of their structure by single-particle cryo-electron microscopy (cryo-EM).

Abstract (up to 10 lines):

With the recent spread of monkeypox infections, poxviruses got into the headlines. We work on the elucidation of structure and function of the poxvirus DNA replication machinery, now mainly by cryo-EM. The DNA replication of vaccinia virus, a safe model system, involves the DNA polymerase holoenzyme complex, built from the polymerase E9, a structural protein A20 and the uracil-DNA glycosylase D4, furthermore the hexameric helicase-primase D5. The long-term aim is to determine structures of these complexes with different bound nucleotides, inhibitors, nucleotide analogues and stabilizing different functional states. The project will build on the team's experiences in the structure determination and biophysical characterization of the partners and their domains and preliminary results on the cryo-EM of DNA-free and DNA-bound complexes. The internship can take a more biochemical, biophysical or computational orientation depending on the profile of the candidate.

Methods (up to 3 lines):

Protein production in the baculovirus-insect cell system or *E. coli*. Affinity chromatography. Characterization of the interaction with DNA substrates by BioLayer Interferometry or Electrophoretic Mobility Shift Assays. Fluorescence–based activity assays. Sample preparation for cryo-EM. Structure calculations.

Up to 3 relevant publications of the team:

Hutin, S., Ling, W.L., Tarbouriech, N., Schoehn, G, Grimm, G., Fischer, U. & Burmeister, W.P. The vaccinia virus DNA helicase structure from combined single-particle cryo-electron microscopy and AlphaFold2 prediction. In preparation.

Tarbouriech N, Ducournau C, Hutin S, Mas PJ, Man P, Forest E, Hart DJ, Peyrefitte CN, Burmeister WP & Iseni F. The vaccinia virus DNA polymerase structure provides insights into the mode of processivity factor binding. Nat Commun. 2017;8: 1455.* doi:10.1038/s41467-017-01542-z

Bersch B, Tarbouriech N, Burmeister WP, Iseni F. Solution structure of the C-terminal domain of A20, the missing brick for the characterization of the interface between vaccinia virus DNA polymerase and its processivity factor. J Mol Biol. 2021; 167009.* doi:10.1016/j.jmb.2021.167009

Requested domains of expertise (up to 5 keywords):

Protein purification, biophysical techniques, computing skills, structural biology