Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

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Title of the M2 research internship:
Preparation of vaccinia virus polymerase complexes bound to DNA and nucleotide substrates

Project summary:
The M2 internship project is part of our research activity on the elucidation of structure and function of the poxvirus DNA replication machinery, principally by single particle cryo-electron microscopy (cryo-EM). The poxvirus family comprises members with a strong potential of spillover from the animal kingdom, such as cowpox and monkeypox. Furthermore poxviruses may be employed in the context of bioterrorism. The structure of the large DNA genome is only shared with asfarviruses and consists of a double-stranded DNA with circularized ends. There is no consensus model for poxvirus DNA replication. The DNA replication of vaccinia virus, a safe model system, involves the DNA polymerase holoenzyme complex, built from the polymerase E9 and a structural protein A20, which forms together with the uracil-DNA glycosylase D4 the processivity factor.
The global aim is to determine structures of the E9-A20-D4 DNA polymerase holoenzyme in complex with different DNA substrates and nucleotides, nucleotide analogues and inhibitors stabilizing different functional states of the polymerase complex. The knowledge of the 3-dimensional structure of these states will help drug design efforts.
The holoenzyme is produced in the baculovirus-insect cell system. A 2-step protocol using 2 different affinity tags allows to obtain the pure complex. Using polyacrylamide gel electrophoresis and fluorescently labelled DNA oligomers, polymerase activity and nucleotide incorporation will be monitored in order to identify interesting states for structural studies by cryo-EM. An additional size-exlusion chromatography step may be used in order to isolate the complexes in different states before grid preparation for cryo-EM. The project will build on the team's experiences in the structure determination and biophysical characterization of several of the central partners and preliminary results on the cryo-EM of DNA-free and DNA-bound forms of the complexes.

Keywords:
DNA replication, virus, single-particle cryo-electron microscopy

Relevant publications of the team:
Burmeister WP, Tarbouriech N, Fender P, Contesto-Richefeu C, Peyrefitte CN, Iseni F. Crystal Structure of the Vaccinia Virus Uracil-DNA Glycosylase in Complex with DNA. J Biol Chem. 2015. doi:10.1074/jbc.M115.648352

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