UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master in Chemistry

Sujet de stage de Master 2 (1 page max.)

Laboratoire : Institut de Biologie Structurale (IBS)
Directeur : Winfried WEISSENHORN

Intitulé de l'équipe : Viral Replication Machines (VRM) Responsable : Marc JAMIN
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Parcours de Master 2 (*Rayer la/les mention(s) inutile(s)*) :

Chemistry for Life Sciences (CLS)
~~Polymers for Advanced Technologies (PTA)~~
~~Organic Synthesis (SOIPA)~~

Titre du sujet :
Structure determination of the DNA polymerase complex of vaccinia virus

Objectifs visés du stage (5 lignes max) :
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 is produced using the baculovirus system and will be studied cryo-electron microscopy (EM) and x-ray crystallography. The production and study by structural NMR and x-ray crystallography of a fragment is also possible.

Intérêts pédagogiques et compétences visées (5 lignes max) :

- Learn the basics in protein expression and purification.
- Discover different biophysical techniques for the study of proteins and protein – ligand interactions.
- Use one of the principal techniques of structural biology: x-ray crystallography, structural NMR and single-particle cryo-EM.

Résumé :
Before its eradication, smallpox has been the most devastating disease of humanity. Nowadays, there is a risk of an introduction of an orthopoxvirus from an animal reservoir into the human population as poxvirus circulate widely at the level of farm animals and wild rodents. The viral DNA polymerase is one of the key proteins for viral replication and is also the target of several antivirals. We determined 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 whose high-resolution structure is still largely unknown, in particular the one of A20. We want to determine the high-resolution structure of the complex by single-particle cryo-electron microscopy or by x-ray crystallography. The structural information on the complex is used in our group in order to design optimized peptides which will interfere with the assembly of the subunits. A collaboration will involve other team of the IBS.

Approches & matériels utilisés (5 lignes max) :
E9-A20-D4 is produced in the baculovirus - insect cell system and purified by affinity and size exclusion chromatography. The structure of the complex will be determined by single-particle cryo-electron microscopy or x-ray crystallography. Components and fragments of the complex can be expressed in *E. coli* and could be studies by NMR and x-ray crystallography.

Domaines de compétences souhaitées du candidat (3 lignes max):

Protein expression, protein purification, structural biology.

Dates du stage : Janvier- Juin 2019