UFR CHIMIE & BIOLOGIE Université Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Departement

Master 2 internship project
Year 2021-2022

Laboratory/Institute: Institut de Biologie Structurale **Director:** Winfried Weissenhorn
Team : Structure and Stability of Intergral Membrane Proteins and Phage Assemblies
Head of team: Cécile Breyton
Name and status of the scientist in charge of the project: Cécile Breyton, DR2 CNRS and Guy Schoehn, DR1 CNRS, head of the EM Platform **HDR :** Yes
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Program of the Master's degree in Biology:
Structural Biology of Pathogens

Title of the project: Determination of the structure of bacteriophage T5 Receptor Binding Protein (pb5) by cryo-EM

Abstract:
Background: Bacteriophages are fascinating nanomachines infecting very specifically bacterial hosts. 60% of them are composed of a capsid, protecting the viral DNA, and a tail, which serves at recognising the host, *via* a Receptor Binding Protein (RBP) located at the tip of its spike. Infection of *E. coli* by bacteriophage T5 is initiated by the irreversible binding of T5 RBP pb5, to its *E. coli* host receptor, the outer membrane transporter FhuA. This interaction triggers the opening of the capsid, the perforation of the host cell wall and the channelling of the DNA to the host cytoplasm. We aim at understanding the conformational changes that occur within pb5 upon binding to FhuA, that are transmitted to the rest of the phage. We are in the process of determining the structure of the FhuA-pb5 complex, by cryo-electron microscopy (cryo-EM). To understand the recognition mechanism, we now need the structure of pb5 before interaction with FhuA.
The project is to obtain the structure of pb5, either purified or within the phage. Pb5 is a 70 kDa protein, *i.e* at the limit of cryo-EM. Thus, the student will explore different approaches. 1- overexpress pb5 and the spike to produce a stable spike-pb5 complex, determine the freezing conditions, screen the cryo-EM grids on the IBS Glacios and if time is available participate in a data collection on a Krios, 2- take advantage of an existing cryo-EM dataset of purified T5 tails, to pick pb5 at the extremity of the tail tip and do image processing to determine the structure of the protein. 3- If necessary, the student will construct a double mutant of T5 leading to tails without the peripherical L fibres. The absence of capsid and fibres will allow optimal grid production and image quality to pick pb5. The project will allow the student to be trained in the very popular cryo-EM technique.

Methods:
Molecular biology, biochemistry, electron microscopy

3 relevant publications of the team:
Linares R, Arnaud CA, Degroux S, Schoehn G, Breyton C. (2020) Structure, function and assembly of the long, flexible tail of siphophages. *Curr Opin Virol*. 45:34-42. doi: 10.1016/j.coviro.2020.06.010. (review)
Arnaud CA, Effantin G, Vivès C, Engilberge S, Bacia M, Boulanger P, Girard E, Schoehn G, Breyton C (2017). Bacteriophage T5 tail tube structure suggests a trigger mechanism for Siphoviridae DNA ejection. Nat Commun. 8(1):1953.
Vassal-Stermann E, Effantin G, Zubieta C, Burmeister W, Iseni F, Wang H, Lieber A, Schoehn G, Fender P (2019) Cryo-EM structure of adenovirus type 3 fibre with desmoglein 2 shows a novel mode of receptor engagement. Nat Commun 10(1).1181

Requested domain of expertise:
Biochemistry and/or structural biology.