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**Title of the M2 research internship:**

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

**Project summary:**

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 its tip. 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- purify pb5, 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 peripheral 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.

**Keywords:**

bacteriophage infection, cryo-EM, virus-host interaction

**Relevant publications of the team:**

Phage structure and characterisation:

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.

Noirclerc-Savoie M, Flayhan A, Pereira C, Gallet B, Gans P, Ebel C, Breyton C (2015). Tail proteins of phage T5: investigation of the effect of the His6-tag position of the His-tag. *Prot Expr & Purif* 109C, 70-78.

Flayhan A, Vellieux FMD, Lurz R, Maury O, Contreras-Martel C, Girard E, Boulanger P, Breyton C (2014). Crystal structure of pb9, the distal tail protein of bacteriophage T5: a conserved structural motif among all siphophages. *J. Virol.* 88, 820-8.

Cryo-EM structure of small and asymmetric macromolecular protein/complex:

Arragain B, Effantin G, Gerlach P, Reguera J, Schoehn G, Cusack S, Malet H. (2020). Pre-initiation and elongation structures of full-length La Crosse virus polymerase reveal functionally important conformational changes. *Nat Commun.* 11(1):3590.

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.

Arragain B, Reguera J, Desfosses A, Gutsche I, Schoehn G, Malet H (2019) High resolution cryo-EM structure of the helical RNA-bound Hantaan virus nucleocapsid reveals its assembly mechanisms. *eLife* 8:e43075.