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

Supervisor(s):
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Host laboratory:
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Host group/team:
M&P

Title of the M2 research internship:

In vitro reconstitution of bacteriophage 9NA with its Salmonella lipopolysaccharide receptor

Project summary:

Background: Bacteriophages are fascinating nanomachines infecting very specifically bacterial hosts. 60% of phages are composed of an icosahedral capsid, protecting the viral DNA, and a tail, crucial for recognising the host, via a Receptor Binding Proteins (RBPs) or Tail Spikes (TSPs) located at its tip. We have determined the structure of E. coli phage T5, before and after interaction of its unique RBP with its receptor FhuA, by electron cryo-microscopy (cryo-EM). This allowed us to elucidate the molecular mechanism of infection trigger, from receptor binding to tail tube opening, membrane anchoring, and outer-membrane (OM) perforation. We are now interested to elucidate this mechanism for another phage, 9NA, which recognises the sugar moiety of lipopolysaccharide (LPS) of Salmonella outer-membrane through six TSPs. Besides this difference, T5 and 9NA share structural similarities in all other tail proteins. We are thus eager to understand the similarities and the differences in the infection mechanism between the two types of phages.
The M2 project is to obtain in vitro conditions to form an active complex between 9NA and its receptor. We achieved this in the case of phage T5 by reconstituting FhuA in a nanodisc, providing the phage with both its receptor and a lipid bilayer. For 9NA, a little patch of LPS will need to be provided. The student will explore different approaches: 1- solubilise Salmonella OM with the amphipathic polymer SMA, 2- use LPS aggregates that can be purified from Salmonella OM and 3- use OM vesicles. The student will check that the purified LPS structure indeed provokes DNA ejection, in particular by negative stain EM, and will optimise the preparation of cryo-EM grids with the most promising sample. Depending on the progress of the project, cryo-EM data may also be collected. This is part of an ANR-DFG project, the student will benefit from the LPS expertise of our German partners.

Keywords:
bacteriophages, infection, cryo-EM

Relevant publications of the team:

1. Linares R, Arnaud CA, Effantin G, Darnault C, Epalle NH, Boeri Erba E, Schoehn G, Breyton C Structural basis of bacteriophage T5 infection trigger and E. coli cell wall perforation. (2023) Sci Adv. Mar 24;9(12):eade9674. doi: 10.1126/sciadv.ade9674.

2. Degroux S, Effantin G, Linares R, Schoehn G, Breyton C. (2023) Deciphering Bacteriophage T5 Host Recognition Mechanism and Infection Trigger. J Virol. Feb 13:e0158422. doi: 10.1128/jvi.01584-22.

3. 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)

4. Arnaud C-A, Effantin G, Vivès C, Engilberge S, Bacia M, Boulanger P, Girard E, Schoehn G and Breyton C (2017) Bacteriophage T5 tail tube structure suggests a trigger mechanism for Siphoviridae DNA ejection Nat Com, 8, 1953 doi: 10.1038/s41467-017-02049-3