Funded PhD project in Integrated Structural & Cell Biology in Grenoble, France (2025-2028)

Title of the PhD project: Does phage T5 form specific viral factories within its host *E. coli* ?

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Host laboratory (UMR): IBS

Host laboratory website: www.ibs.fr

Host team/group: M&P and MEM

Team website, if any: https://www.ibs.fr/en/research/membrane-proteins-and-glycobiology/membrane-and-pathogens-group-f-fieschi/structure-and-stability-of-integral-membrane-protein-and-phage-assemblies-team/?lang=en and https://www.ibs.fr/en/research/assembly-dynamics-and-reactivity/methods-and-electron-microscopy-group-g-schoehn/schoehn-team/

Project summary: Bacteriophages are fascinating and remarkably efficient nanomachines. These viruses of bacteria specifically recognise their host with their tail, that then perforates the bacterial cell wall and inject the viral DNA, which is densely packed in the viral capsid, in their prey's cytoplasm. In the lytic cycle, the phage DNA then takes over the host cell metabolism and machinery to produce new virions, which are released at the end of the cycle by lysis of the bacterium. We know from biochemical and genetic studies that the phage capsid and the tail are assembled independently, but how is the cell reorganised to ensure its new viral factory role? The time is ripe, with tremendous instrument and software improvements, to investigate what happens directly into the infected cell. The project aims at imaging the cytoplasm of infected cells, at different time points of the lytic cycle, by cryo-electron tomography of cryo-FIB/SEM milled lamellas. This will allow the student to unravel the spatial and temporal reorganisation of the host turned into a viral factory. We will use bacteriophage T5 and its host *E. coli* for this study, as we have in hands a number of mutants, blocked at different stages of the lytic cycle and a great expertise in its structural study.

Student role: The student will perform all experiments of the project, from phage T5 amplification *E. coli* infection optimisation, classical plastic embedding cellular EM and finally sample freezing (conventional plunge freezing and high-pressure freezing) of T5-infected bacteria, cryo FIB/SEM lamella preparation and screening on the Glacios to find the best experimental condition, image analysis of tomography series acquired using a Titan Krios and data interpretation.

Skills/Qualifications: Phage microbiology and/or electron microscopy sample preparation techniques and imaging methods. Enthusiasm and dedication.

Keywords: Bacteriophage, cryo-FIB/SEM, electron tomography, lytic cell cycle

Relevant publications of the team: (5 max.)

Linares R and Breyton C (2024) About bacteriophage tail terminator and tail completion proteins: structure of the proximal extremity of siphophage T5 tail . *J Virol.* 2024 Dec 23:e0137624. doi: 10.1128/jvi.01376-24.
Alexander LT… Breyton C… Degroux S… Schwede T. (2023) Protein target highlights in CASP15: Analysis of models by structure providers. Proteins. doi: 10.1002/prot.26545.
Linares R, Arnaud CA, Effantin G, Darnault C, Epalle NH, Boeri Erba E, Schoehn G, Breyton C. (2023). Structural basis of bacteriophage T5 infection trigger and *E. coli* cell wall perforation. Sci Adv. 9(12):eade9674.
Degroux S, Effantin G, Linares R, Schoehn G, Breyton C. (2023) Deciphering Bacteriophage T5 Host Recognition Mechanism and Infection Trigger. J Virol. 2023 97(3):e0158422.
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.