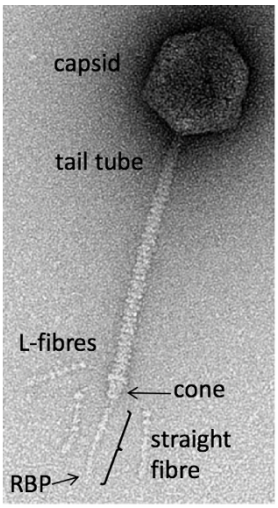
ESRF	**Experiment title:** Structure of the tail tip complex of phage T5	**Experiment number**: MX-2031
Beamline: CM01	**Date of experiment**: from: 02/02/2018 to: 05/02/2018	**Date of report**: 10/09/2018
Shifts: 3 days	**Local contact(s)**: Grégory Effantin	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):
Cécile Breyton*, Institute for Structural Biology
Romain Linares*, Institute for Structural Biology

Report:



We are working on bacteriophage T5, a virus infecting *Escherichia coli* (Fig. 1). The T5 virion is made of a capsid containing the viral DNA and a tail, a large protein complex involved in virus adsorption to the bacterial host, perforation of the cell wall and safe channeling of the viral DNA from capsid to the host cytoplasm.
We are currently working on the Tail Tip Complex (TTC), the extremity of the tail that bears the receptor binding protein and that drastically changes conformation, to perforate the cell-wall. The first step is to determine its structure before the interaction with the receptor.

Figure 1: Negative stain electron microscope of phage T5. Diameter of the capsid: 90 nm. The distal part of the tail forms the Tail Tip Complex, which is complosed of the cone and the straight fiber, at the extremity of which is located the Receptor Binding Protein (RBP).

Data collection was successful, with 3208 images aquired during the 3-days allocated. We had to screen many grids to get one with the right ice thickness, even though we had checked that grids should have been ok on one of 12. This grid resulted from a rather dirty sample (Fig. 2). Collected images were of good quality, except for 421 images that were either too close to focus or had too thick ice. From the good images, 10550 particles were manually picked with helix boxer and treated with Relion.

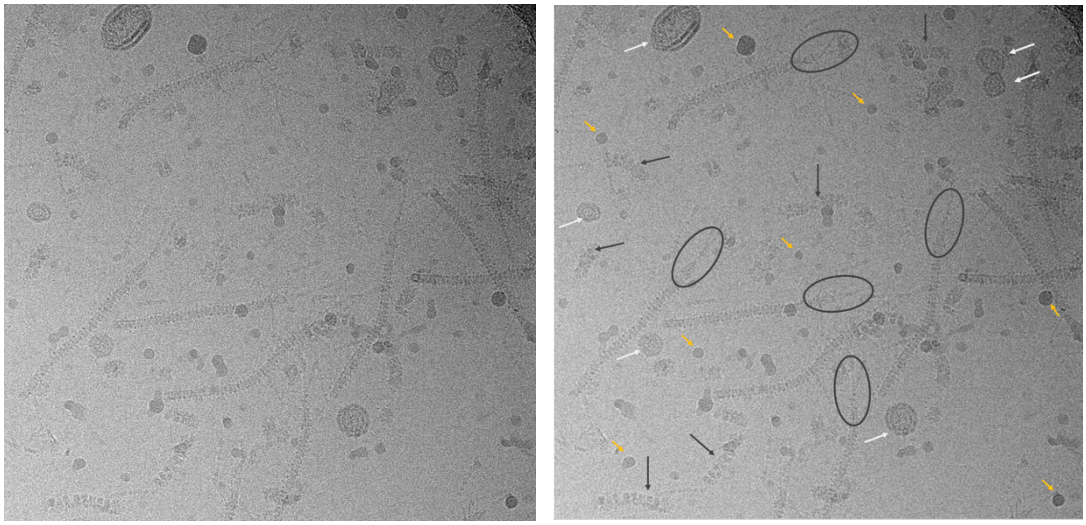


Figure 2: Typical collected image, showing the tails of phage T5. Left: raw image, right: circled dark gray: T5 TTC; dark gray arrows: protein contaminant; white arrows: vesicle contaminants; yellow arrows: ice contamination.

The first 2D class show data to 4Å resolution (Fig. 3), suggestive of a 3D structure to better resolution.

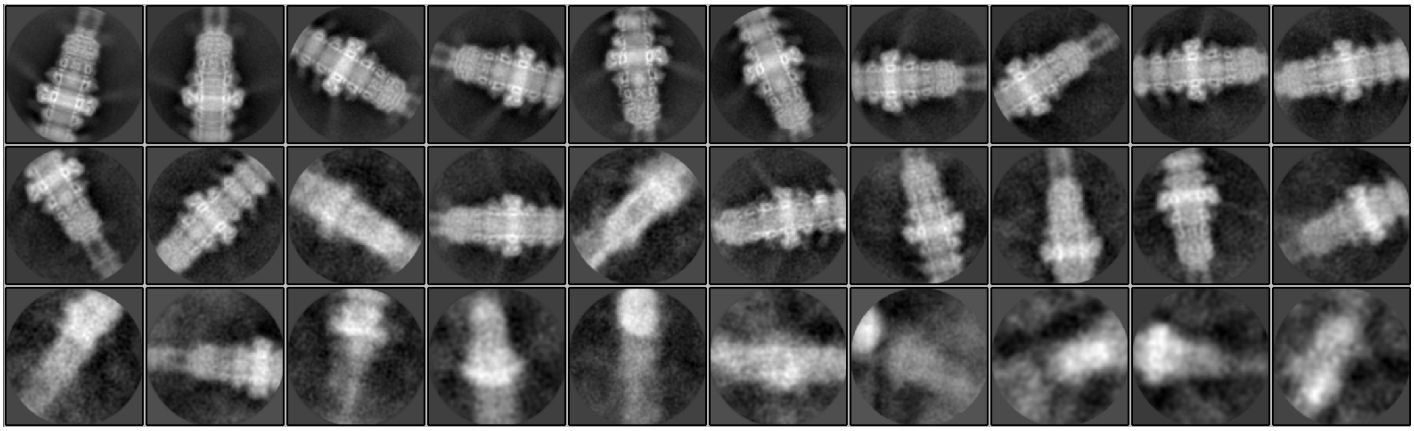


Figure 3 : 2D classes of the manually picked particles. The first two lines of classes, containing the majority of the particles (8710 particles).

3D reconstruction, centered on the cone of the TTC, located just under the collar from which the L-fibers start, shows a map of extremely good quality, to a resolution of 3.48Å after three fold symmetry averaging. Structures of individual proteins determined by crystallography fit remarquably well (Fig. 4). For those proteins of unknown structure, the map is of sufficiently good quality to trace the chain and determine the structures *de novo*. From the same images, but changing the position of the center of the box used for picking the particles, structure of the fiber will also be possible to determine, probably to lower resolution.

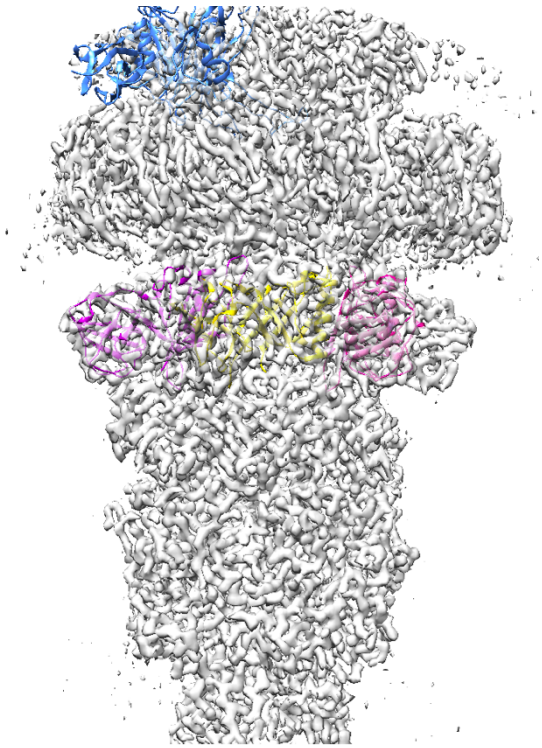


Figure 4: 3D structure of the upper part of the TTC. In blue, ribbon representation of the structure of the monomer of pb6 (fitting is not straightforward here because the pb6 ring is truncated), in pink, yellow and magenta, ribbon representation of the monomer of pb9 fitted in the density of pb9 hexamer. Map quality is good enough to trace *i*- pb3 trimer (below pb9), *ii*- p140 trimer, between pb9 and pb6 and *iii*- p132 and/or parts of pb1 forming the collar surrounding p140… A lot of fun ahead!