Structural Analysis of *Listeria* Phage A511 Baseplate by Cryo-Electron Microscopy and Cryo-Electron Tomography

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Contractile tailed bacteriophages of Gram-positive bacteria are among the largest and most structurally complex phages. Yet, the structure of their host adsorption apparatus – the contractile tail and complex baseplate – has never been investigated in detail. We report here the structure of the broad species-specific *Listeria* phage A511 in its pre- and post-host attachment states by cryo-electron microscopy and tomography (cryo-EM and cryo-ET).

*L. ivanovii* was used as the host for phage A511 propagation. A511 baseplate contraction was achieved through 1 hour dialysis against 2 M urea. *L. monocytogenes* peptidoglycan and teichoic acids were isolated as previously described [1] and phages were allowed to attach for 5 minutes. A511 particles and phage-infected peptidoglycan were flash frozen onto glow-discharged Quantifoil carbon grids in liquid ethane with an FEI Vitrobot. Cryo-EM single particle and tomography data were collected with an FEI Tecnai F20 on an Eagle 4k x 4k CCD camera.

As observed in phages of Gram-negative bacteria, the distal end of the A511 tail tube carries the central spike protein (gp99) that extends from the baseplate hub protein gp98, which contains a peptidase domain. This complex was not observed in the cryo-EM map of the contracted tail. The A511 baseplate contains twelve copies of a large, 3x128 kDa, trimeric protein that is likely encoded by gene 106. Gp106 is a VrlC-like protein previously found in phages of Gram-positive and Gram-negative bacteria with a putative function of binding to host cell receptors [2, 3]. These proteins undergo a large amount of structural rearrangement associated with sheath contraction: 6 copies of gp106 rotate about 180 degrees to face the host membrane (Fig. 1). To this end, the transformation of the A511 baseplate resembles that of the lactococcal phage p2 [4].

A511 sheath contraction starts from the baseplate and we were able to capture and visualize intermediates of this process by cryo-ET (Fig. 2). Quantitative analysis of cryo-ET data (Fig. 3) revealed a particle population with partially contracted tails and an average tail sheath length of 149.2 nm, in addition to the 103.9 nm contracted sheaths and the 194.0 nm extended ones.

References:
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Figure 1. Density map depicting the VrlC-like protein gp106 in the extended and contracted A511 baseplate. One gp106 complex was computationally extracted and rotated to illustrate the structural modifications that take place during contraction.

Figure 2. Averaged 9.2 nm tomographic slices (top) and diagrams (bottom) illustrating the structural rearrangement of A511 phage tail upon attachment to L. monocytogenes cell walls. A. unattached phage; B. initial attachment with long tail fibers; C. baseplate contraction with gp106 rotation and partial sheath contraction; and D. fully contracted phage.

Figure 3. Histogram of A511 sheath length after 5-minute incubation with L. monocytogenes cell walls. The 3 peaks represent the 103.9 nm contracted sheaths (SD=3.7 nm; n=80), the 149.2 nm partially contracted sheaths (SD=10.6 nm; n=20) and the 194.0 nm extended sheaths (SD=8.3 nm; n=67).